Friedrich-Schiller-Universität Jena

IMPRS
Exploration of Ecological Interactions with Molecular and Chemical Techniques

Chemical communication in an aphid-natural enemy system: new mechanisms of aphid alarm signalling and wing induction

Dissertation
Zur Erlangung des akademischen Grades
doctor rerum naturalium (Dr. rer. nat.)

vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät
der Friedrich-Schiller-Universität Jena

von Master of Science (M.Sc.) in Applied Biosciences
Eduardo Hatano
geboren am 02.07.1980 in São Bernardo do Campo, Brazil

Gutachter

1) Prof. Dr. Wolfgang W. Weisser – Friedrich-Schiller-Universität

2) Prof. Dr. Jonathan Gershenzon – Max-Planck-Institut für chemische Ökologie

3) Prof. Dr. Yannick Outreman – INRA-Agrocampus Ouest-Université Rennes

Tag der öffentlichen Verteidigung:

18 Januar, 2010

Table of contents

1. INTRODUCTION

Communication is the act of conveying information from one organism, the signaller, to another, the receiver, and elicits specific behavioural or physiological or morphological responses from the latter (Théry & Heeb, 2008). It is essential for organisms living in colonies to share the collected information of the surrounding habitat with conspecifics to predict risks and opportunities, and coordinate the group to enhance direct and/or indirect individual fitness (Fletcher, 2007; Huang & Robinson, 1992). Therefore, by mediating and modifying the behaviour of an individual, communication strongly affects the evolutionary and population ecology of species (Dicke & Grostal, 2001). In many circumstances, the social interactions of the group, and therefore communication among individuals, may mediate the division of labour and phenotypic plasticity within a colony (Huang & Robinson, 1992; Robinson, 1992).

A signal may convey different ecological information, e.g. the availability of resources (Alcock, 1998a; Wright & Schiestl, 2009) or shelter (Visscher, 2007), the presence of potential sexual mates (Alcock, 1998c; Cardé & Baker, 1984), and the population density (de Kievit & Iglewski, 2000). Signalling the presence of a predator for synchronization of defence is a central trait necessary for the evolution of other traits (Pike & Foster, 2008), because predation causes more serious, immediate and direct fitness costs than do other factors, e.g. starvation (Inman & Krebs, 1987; Lima & Dill, 1990). Alarm signalling is one strategy that evolved in many animal species to alert conspecifics and thereby reduces their risk of being preyed on. Signals can be of many kinds: acoustical (Hollen & Radford, 2009; Kelley, 2004), visual (Brown *et al.*, 1999) or chemical (Harborne, 1993; Law & Regnier, 1971), and may have two modes of action for the preys. First it can alert conspecifics which may react with escaping (Alcock, 1998b), thanatosis so as to be undetected (Miyatake *et al.*, 2009), hiding in shelters (Venzon *et al.*, 2000), or attacking (Rhoden & Foster, 2002). The signaller may also benefit from mutualistic interactions and rely on protection from interspecific organisms (Fiedler *et al.*, 1996; Flatt & Weisser, 2000). Second it can directly deter the predator attack (Ruxton *et al.*, 2004).

Chemical compounds play an important role in mediating the communication of cells, tissues, multicellular organisms and finally groups of individuals. Because compounds may have different structures and traits and there are many environmental influences, species or groups of taxonomically related species, communicate using one or a few stereotyped compounds. Volatile organic compounds, for instance, are highly lipophilic products of low molecular weights that are important for relative long-distance communication especially for insects and plants (Tholl *et al.*, 2006). Insects make use of volatile compounds as alarm pheromones to alert conspecifics of the presence of predators. The chemical structures of alarm pheromones vary greatly among species.

If a signal were perceived by a non-target insect it could have high costs to signallers and original receivers (Blum, 1969; Mustarpa, 1984). However, insect chemoreceptors and odorant-binding proteins in insect antennae can differentiate specific structures from other similar structures or isomers (Matsuo *et al.*, 2007; Pelosi *et al.*, 2006; Xu *et al.*, 2005). In addition, some insect species may use more than one compound and a certain optimal ratio among the

compounds that trigger the alarm behaviour, while single compounds may cause no or little response (Bruce *et al.*, 2005). These two traits, alone or combined, may assist insects in reliably discriminating the relevant alarm pheromones from compounds of other sources and is especially relevant for alarm pheromones, since among all pheromones, they are the least specific compounds (Blum, 1969).

If a species use more than one system to avoid predators when alarmed, it may optimize its strategy by reallocating their resources to different types of responses to trade-off the benefits and risks (Dicke & Grostal, 2001; Kats & Dill, 1998). Insects respond to alarm pheromones in various ways: some may initiate a defence, some may disperse or keep feeding depending on other factors, e.g. the presence of competitors, mates, natural enemies, climate conditions, the availability of resources and previous experiences (Dicke & Grostal, 2001; Tollrian & Harvell, 1998). Aphids, for instance, are highly dependent on their alarm pheromones to survive an imminent predator attack. A remarkable characteristic of aphids is their phenotypic plasticity: aphids can produce individuals with different morphologies according to different stimuli, including the emission of alarm pheromones and, therefore, the presence of a natural enemy. Because all offspring produced by parthenogenesis are clones of their mothers and exhibit different polyphenisms, aphids are an ideal organism for studying the influence of external factors on phenotype while excluding genetic variation. However, the morphological, physiological and behavioural responses of aphids when alarmed cannot be generalized because they vary among and within species according to the ecology of each individual.

1.1. Aphids: life cycle, reproduction and morphs

Aphids (Order Hemiptera; Suborder Sternorryncha) are small (1-10mm), soft-bodied insects of different colours and morphs. They feed on the phloem of plants using piercing-sapping stylets as mouthparts (Klingauf, 1987). They possess antennae with five or six segments with two basal segments and a segmented flagellum, a pair of tube-like structure called siphunculi on their fifth abdominal segment, and a cauda that releases droplets of honedew from the anus (Blackman & Eastop, 2007).

The life cycles of aphids can be divided into two types according to their host range: a host specific cycle (autoecious, Fig. 1) and a host alternating cycle (heteroecious, Fig. 2). Autoecious aphids feed and reproduce on one or a few species of a genus during their life cycle. Heteroecious aphids, on the other hand, live on woody plants (primary host) during autumn, winter and spring and then migrate to herbaceous plants (secondary plant) where they live in the summer. These include 15% of the species of the subfamily Aphidinae, which is the largest subfamily and which contains most of the agriculturally important species (Blackman & Eastop, 2007). Both life cycles are, however, similar in their alternation of reproduction along the seasons with different morphs along their cycles. Diapausing eggs are laid on primary hosts where they overwinter. In spring, aphids (fundatrix) hatch and give birth to females. During summer, aphids reproduce by parthenogenesis, which means eggs develop inside the female's body without endomeiosis or internal chromosomal recombination (Blackman, 1987; Hales *et al.*, 2002).

Aphids' lifespans are brief and within a few days they become adults. Because of shorter days in autumn, parthenogenetic aphids produce sexupara, which give birth to males and ovipara females, switching to sexual reproduction (Lees, 1966). The sexual morphs then lay the diapausing eggs, resetting the life cycle. Heteroecious aphids differ from autoecious aphids in their production of migrant morphs in spring and autumn when moving between primary and secondary hosts (Fig. 1 and 2).

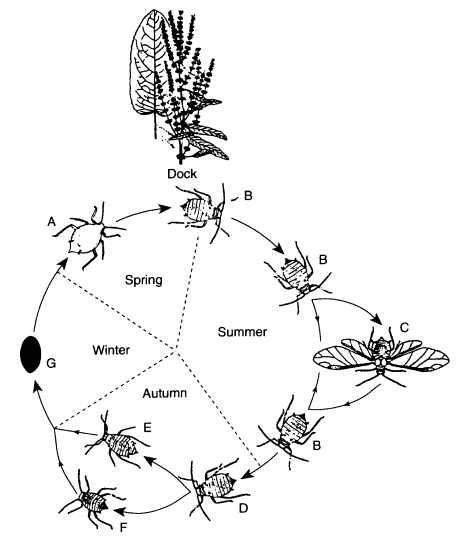

Figure 1. Autoecious life cycle. (A) Fundatrix, (B) unwinged morph, (C) winged morph, (D) sexupara, (E) mating female, (F) male, (G) egg (Dixon, 1998)

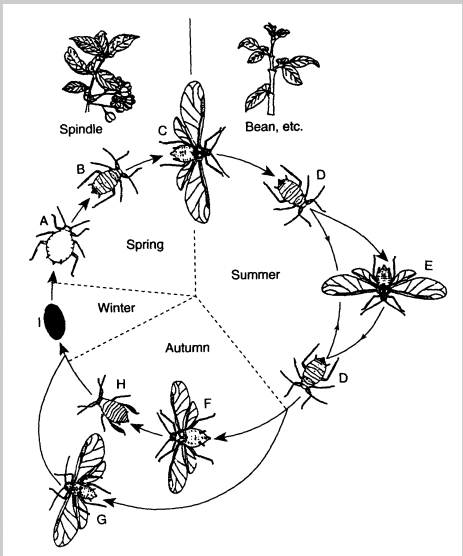

Figure 2. Heteroecious life cycle. (A) Fundatrix, (B) fundatrigenia, (C) emigrant, (D) unwinged morph, (E) winged morph, (F) gynopara sexupara, (G) male, (H) mating female, (I) egg (Dixon, 1998)

1.2. Natural enemies and defences of aphids

Because of their high abundance and ubiquitous distribution, aphids are attacked by a wide range of natural enemies. These include not only specialized parasitoids such as aphid wasps (Hymenoptera: Aphidiinae), but also predators such as ladybirds, lacewings, hoverflies, anthocorid bugs, spiders, carabid beetles or even birds. Microbes also play an important role as the natural enemies of aphids, especially pathogenic fungi. However, they are often forgotten when analysing the tri-trophic interactions that include aphids (Roy & Cottrell, 2008).

Because aphids are sedentary insects, they have very effective defensive systems. When aphids are disturbed or attacked, they attempt to kick, walk away, drop from plants and release cornicle droplets from a pair of elongated abdominal structures called siphunculi (Fig. 3) (Dixon, 1958). These cornicle droplets contain a mixture of fatty acid radicals and alarm pheromone. They are stored in cornicle sacs under the siphunculi in secretory cells called oenocytes; these are surrounded by a fluid whose content is probably the same as that from ruptured cells (Chen &

Edwards, 1972; Edwards, 1966). When the aphid is attacked, the cornicle droplets are emitted by turgor pressure of the aphid abdomen under control of the nervous system (Edwards, 1966; Strong, 1967). The mixture of the fatty acid radicals hexanoyl, sorboyl, myristoyl, palmitoyl (Greenway & Griffith.Dc, 1973; Greenway *et al.*, 1974) rapidly crystallize when in contact with the surface of predators and parasitoids, halting their attack and releasing the aphid (Dixon, 1958; Edwards, 1966).

Figure 3. The aphid cornicle droplet.
When pea aphids are seized by a natural enemy, in this case a third-instar larvae of *Chrysoperla carnea*, they emit cornicle droplets; these contain a mixture of fatty acid radicals and the alarm pheromone, (*E*)-β-farnesene.

The alarm pheromone volatilises from the droplets and warns neighbouring conspecifics of the presence of natural enemies (Kislow & Edwards, 1972; Nault *et al.*, 1973). When the pheromone is perceived by the rhinaria (specialised structures on the aphids' antennae) (Wohlers & Tjallingii, 1983), aphids respond by withdrawing their stylets, dropping off the host plant, moving to other parts or plants (Kislow & Edwards, 1972; Nault *et al.*, 1973; Nault & Phelan, 1984; Roitberg & Myers, 1978; Wohlers, 1980), or simply squirming (Nault *et al.*, 1976). Since the alarm pheromone is emitted only when an aphid is attacked, it signals nearby aphids to reallocate to sites where there is less risk of encountering a natural enemy. However, in a few species that produce soldiers, they become aggressive towards the threat (Arakaki, 1989).

The sesquiterpene (*E*)-β-farnesene was primarily identified as the alarm signal commonly used among four aphid species (*Acyrthosiphon pisum*, *Macrosiphum rosae*, *Schizaphis graminum* and *Aphis gossypii*), and was likely involved in other six species (Bowers *et al.*, 1972). Edwards *et al.* (1973) and Wientjen *et al.* (1973) supported the finding that (*E*)-β-farnesene was widespread within different aphid species. However, the biosynthesis of (*E*)-β-farnesene in aphids is still unknown. Later, other compounds were detected in the blend of aphid alarm pheromone (Nault & Bowers, 1974; Pickett & Griffiths, 1980). Francis *et al.* (2005) published a table with all compounds contained in the alarm pheromone blend from 23 aphid species, in which 13 had (*E*)-β-farnesene as the only compound, including *A. pisum*, 3 had it as the main compound, 5 had it in minute amounts and the remaining 2 did not have (*E*)-β-farnesene in their blend. The other compounds include different terpenes, as for example, germacrene D, (*Z,E*)-α-farnesene, (*E,E*)-α-farnesene, limonene, and α- and β-pinene (Francis *et al.*, 2005; Pickett & Griffiths, 1980), and isothiocyanates (Francis *et al.*, 2005).

1.3. Ecology of aphid alarm pheromones

Although (*E*)-β-farnesene emission is beneficial for aphids by relaying information to conspecifics about the presence of a natural enemy, it also reveals the presence of aphids to other organisms. Many insect species have evolved the ability to perceive this signal and use it to locate aphids. Nault *et al.* (1976) was the first to present evidence that the aphid alarm pheromone was involved in interspecific communication, specifically, the recruitment of the aphid-tending ant, *Formica subsericea*, for protection. This ability to perceive (*E*)-β-farnesene (and other aphid-related cues) to locate and recognize aphids on plants is also widespread among many natural enemies such as the coccinelid, *Adalia bipunctata* (Francis *et al.*, 2004), the lacewing, *Chrysoperla carnea* (Zhu *et al.*, 1999), and the parasitoid, *Lysiphlebus testaceipes* (Micha & Wyss, 1996). In addition, there are also fitness costs for signallers and perceivers attributed to emitting this compound. For instance, third and fourth instars nymphs of *A. pisum* that were artificially disturbed to release cornicle droplets delayed the production of offspring (Mondor & Roitberg, 2003). In another study, *A. gossipy* that were exposed to (*E*)-β-farnesene when they were first instar nymphs had longer developmental time, lower fecundity and weight when they became adults (Su *et al.*, 2006).

Since it is costly to stop feeding and escape involves the risk of predation (Dill *et al.*, 1990), aphids can respond differently to alarm pheromones. There is substantial intra- and interspecific variation in the behavioural responses of aphids to alarm pheromone. For example, first instar nymphs of *A. pisum* (Roitberg & Myers, 1978), *Myzus persicae* (Montgomery & Nault, 1978) and *Diuraphis noxia* (Shah *et al.*, 1999), which bear a high risk of starvation when alarmed, are less sensitive to alarm pheromone than adults. Different morphs of aphids also behave differently: in general, winged morphs and soldiers are more sensitive to alarm pheromones than other morphs (Arakaki, 1989; Rhoden & Foster, 2002; Uematsu *et al.*, 2007; Visser & Piron, 1997), probably because morphological selection also favoured high sensitivity to (*E*)-β-farnesene. In addition, different aphid clones, which may vary in colour type and be adapted to feed on different host plants or avoid some natural enemies, also adopt different alarm behaviours (Kunert *et al.*, 2010). As for species, aphid clones are under different selective pressures and, therefore, it is likely that different alarm responses of clone types are related to their habitats. In fact, extrinsic factors, such as the low abundance of host plants (Montgomery & Nault, 1977), the high nutritional quality of host plant and dry environment (Dill *et al.*, 1990), strongly inhibit the dropping behaviour of aphids because of the high risks of desiccation and starvation. The alarm behaviour may also depend on the species of natural enemy. For instance, the predatory gallmidges *Aphidoletes aphidimyza* and *Leucopis annulips* feed on aphids but do not trigger the production of cornicle droplets (Frechette *et al.*, 2008; Lucas & Brodeur, 2001). This strategy benefits the larvae because it reduces the costs of foraging for another aphid.

1.4. Wing induction in parthenogenetic aphids

Under adverse conditions during the parthenogenetic part of the life cycle, aphids can produce winged offspring which disperse to colonise new host plants. It allows aphids to quickly adapt to and exploit different habitats while preserving their genotype. This alternation of phenotypes can be maternally controlled before nymphs are born (Sutherland, 1967), or it can also be determined during postnatal nymphal stages; this is true for *Aphis fabae* (Shaw, 1970b) and *Aphis craccivora* (Johnson & Birks, 1960).

Crowding is one of the factors that induce wing formation; when populations are overcrowded, aphids reduce competition levels by migrating to other hosts. Previous experiments showed that aphids reared in isolation or at low population densities did not produce winged offspring, while aphids reared at high densities triggered the production of winged morphs. This was shown for many aphid species such as *Megoura viciae* (Lees, 1967), *A. pisum* (Sutherland, 1969), *A. fabae* (Shaw, 1970a) and *M. persicae* (Sutherland & Mittler, 1971). Therefore, constant physical contact among aphids is thought to stimulate the production of winged offsprings which varies inter- and intraspecifically with some aphids producing very few winged offspring even at very high densities and vice versa (Sutherland, 1969). The mechanical stimuli are perceived by tactile hairs on the body, legs, and antennae of the aphid mother and trigger the wing induction (Sutherland, 1969). However, winged *A. pisum* and *A. fabae* are less responsive to crowding because they produce little or no winged offspring (Shaw, 1970a; Sutherland, 1970).

Poor quality of host plant or parts of plant may have an independent effect or work synergistically with crowding to induce wing formation depending on the aphid species. For example, *A. pisum* that were kept in isolation and fed old leaves of *Vicia faba* produced a higher proportion of winged offspring than did pea aphids fed with young leaves (Sutherland, 1967). It did have a significant effect on *Aphis craccivora* (Johnson, 1966) and *Megoura viciae* (Lees, 1967) when combined with crowding to trigger wing induction, but not alone. It is likely that the interaction between both factors is due to the fact that aphids feeding on low quality plants or parts of plants are less rested and tend to move more, increasing their contact rate.

Temperature and photoperiod also play a role in wing induction. High temperatures and long days had a negative effect on wing induction in *A. craccivora* and *M. Viciae*, but the effect was reversed with crowding and host quality (Hodgson *et al.*, 2005; Johnson, 1966; Lees, 1967).

The presence of other organisms can also affect the aphid wing induction. Mutualistic interactions with ants, for instance, tend to inhibit aphid movements and wing formation, and are likely related to the protection ants provide to aphids in exchange for a supply of honeydew (Nault *et al.*, 1976). Chemical compounds from the ant *Lasius niger*, a mutualistic ant for *A. fabae*, limited the dispersal of this aphid when they were applied in a filter paper but had no effect on *A. pisum*, a species which is not ant tended (Oliver *et al.*, 2007). Limited mobility might reduce the contact among *A. fabae* and explain why they produce fewer winged offspring in the presence of ants. In addition, mutualistic ants may apply compounds such as dendrolasin from their mandible glands that inhibit the physiology of wing induction in female aphids (Kleinjan & Mittler, 1975).

Contrary to the effect of mutualistic ants, the presence of natural enemies triggers the aphid wing induction. After the observations that the presence of predatory ladybirds and their tracks

increased the proportion of winged offspring in colonies of *A. pisum* (Dixon & Agarwala, 1999; Weisser *et al.*, 1999), the same phenomenon was observed for other predators, such as the lacewing *C. carnea* and larvae of the hoverfly *Episyrphus balteatus* (Kunert & Weisser, 2003) and for the parasitoid *Aphidius ervi* (Sloggett & Weisser, 2002). Because these natural enemies had different modes of attack, it was suggested that the aphid wing induction caused by natural enemies was mediated by a general mechanism: the alarm behaviour caused by the emission of alarm pheromone. Kunert *et al.* (2005) demonstrated that colonies treated with alarm pheromone produced a higher proportion of winged offspring even at low population densities than undisturbed colonies. Interspecific variation in this mechanism was then observed for different clones of *A. pisum* that produced different proportions of winged morphs when exposed to (*E*)-β-farnesene (Schwartzberg *et al.*, 2008a). Other aphid species such as *Megoura vicea* and *Aphis fabae* do not produce more winged morphs when exposed to natural enemies (Kunert *et al.*, 2008).

1.5. Aims and questions

Thus (*E*)-β-farnesene mediates many interactions and there is much variability in alarm behaviour which is dependent on the ecology of aphid species. However, I) the consequences of (*E*)-β-farnesene to organisms other than aphids and II) the proximal factors that influence the signalling process are still poorly explored in the interactions mediated by alarm pheromone.

I) While it is known that (*E*)-β-farnesene is perceived by the natural enemies of aphids, this signal is unlikely detectable at long distances because aphids emit low amounts. Therefore, we still lack the information of how this signal is perceived by different species and what differences it causes in the foraging strategies of predators and parasitoids. Interestingly, some natural enemies interact with aphids but do not cause aphids to emit the cornicle droplet making the role of (*E*)-β-farnesene in the interaction of aphids and natural enemies more complex.

II) While many studies focus on the ultimate consequences of alarm behaviour for aphids, there is little attention paid to proximate factors that influence the aphid alarm signalling. Similar selective pressures that caused the variations in alarm behaviour may have also determined the optimal signalling for aphids to flexibly adapt to different conditions. For example, species of natural enemies (Lucas & Brodeur, 2001), aphid age (Mondor *et al.*, 2000), morph type (Gut & Vanoosten, 1985) and group size (Verheggen *et al.*, 2009) are some of the factors that change the emission of alarm pheromone by aphids.

In order to better understand the role of (*E*)-β-farnesene as the alarm pheromone of pea aphids in natural ecosystems, these two factors, alarm signalling and different natural enemies, need to be further studied.

Overall question

How does the aphid alarm pheromone, (*E*)-β-farnesene, shape and how is it is shaped by, aphid-natural enemies and aphid-aphid interactions?

Questions

1. What are the ecological consequences of alarm signalling for aphid colonies?
2. What is the role of (*E*)-β-farnesene for the natural enemies of aphids?
3. Can aphids regulate their emission of alarm pheromone? What factors affect this regulation?
4. Do entomopathogenic fungi also induce wing formation in aphids via the pseudo crowding effect as arthropods' natural enemies do?

1.6. Overview of Manuscripts

Manuscript I

Aphid wing induction and ecological costs of alarm pheromone emission under field conditions (2010)
Hatano E., Kunert G., Weisser W.W. Accepted in *PLoS One*.

This manuscript deals with the pseudo crowding effect on aphid wing induction by testing the indirect effect of (*E*)-β-farnesene under natural environmental conditions. In field tests, aphid colonies exposed to (*E*)-β-farnesene produced a higher proportion of winged offspring than did control colonies, even when the population density was low. This is the first report that demonstrated the effect of (*E*)-β-farnesene on wing induction under field conditions. Furthermore, this response also indicates a cost of alarm signalling since it reduces the survival of neighbouring aphids. This manuscript addresses question 1.

E. Hatano performed the experiment and together with W.W. Weisser wrote the manuscript. G. Kunert designed the experiment, and helped with statistical analysis and edition of the manuscript.

Manuscript II

Chemical cues mediating aphid location by natural enemies (2008)
Hatano E., Kunert G., Michaud J.P., Weisser W.W. *European Journal of Entomology* 105, 797-806.

This manuscript reviews how predators and parasitoids locate their host aphids using semiochemicals from plants or aphids or a combination of both. The information is organized according to a stepwise location process. Volatiles from plants attacked by aphids play a main role in directing natural enemies at long range, followed by volatile cues from the aphids or their honeydew to guide natural enemies at short range. Host acceptance, the identification of a suitable host, is then triggered by contact kairomones of the aphid cuticle. This manuscript complements the previous manuscript to answer question 1.

E. Hatano wrote the manuscript together with G. Kunert and W.W. Weisser. J.P. Michaud corrected and gave critical comments on the manuscript.

Manuscript III

Aphid alarm pheromone mediates avoidance of habitats with increased risk of intraguild predation Hosseini M., **Hatano E.**, Ashouri A., Weisser W.W. Submitted on 22 July 2009 to *Ecological Entomology*.

This manuscript addresses the third question and studies the role of (*E*)-β-farnesene as a signal for the presence of potential intraguild predators for *A. aphidimyza*. When exposed to (*E*)-β-farnesene, gallmidge larvae reduce their foraging behaviour and leave the host plant. The increase in head circulation movements of larvae indicate that the larvae can perceive the signal. These results suggest that (*E*)-β-farnesene from aphids is used to reduce the risk of intraguild predation to gallmidge larvae.

This experiment was designed by M. Hosseini, E. Hatano and W.W. Weisser. It was conducted by M. Hosseini. E. Hatano assisted and supervised the experiment. W.W. Weisser wrote the manuscript. E. Hatano, M. Hosseini and A. Ashouri corrected and commented on the manuscript.

Manuscript IV

Do aphid colonies amplify their emission of alarm pheromone? (2008)
Hatano E., Kunert G., Bartram S., Boland W., Gershenzon J., Weisser W.W. *Journal of Chemical Ecology* 34, 1139-1142.

This manuscript addresses the fourth question and examines whether there is a signal cascade in which aphids that perceive (*E*)-β-farnesene but are not attacked respond by emitting alarm pheromone. The results indicate that colonies of pea aphid exposed to a synthetic deuterated (*E*)-β-farnesene do not release any (*E*)-β-farnesene. It suggests that pea aphids can perceive very low amounts and remain therefore inconspicuous by not emitting large amounts of (*E*)-β-farnesene.

E. Hatano conducted the synthesis of deuterated (*E*)-β-farnesene, which was designed by S. Bartram. E. Hatano conducted the bioassays, analysed data and wrote the manuscript. G. Kunert, S. Bartram, W. Boland, J. Gershenzon and W.W. Weisser corrected and commented on the manuscript. The idea of this experiment was conceived by G. Kunert.

Manuscript V

Don't talk so loud: the emission of aphid alarm pheromone regulated by social conditions
Hatano E., Kunert G., Kunert M., David A., Gershenzon J., Weisser W.W. In preparation for publication in *Ecological Entomology*.

This manuscript continues exploring question 4 by investigating the effect of aphid population density on the production and emission of alarm pheromone. The amount of (*E*)-β-farnesene emitted by two aphid clones reared under different group conditions when attacked by lacewing larvae is assessed. The amounts of (*E*)-β-farnesene from grouped aphids are lower than aphids that were isolated. However, the production of (*E*)-β-farnesene is not affected by either condition. Furthermore, the pea aphid clones show different dispersal and developmental strategies according to colony size and emission of alarm pheromone. The amounts of (*E*)-β-farnesene detected from isolated and grouped aphids are tested in aphid colonies and show that the regulation of (*E*)-β-farnesene emission according to population densities adaptively induces wing formation in offspring.

E. Hatano designed and conducted this experiment, analysed data and wrote the manuscript. M. Kunert and A. David assisted in the volatile collection. G. Kunert, M. Kunert, A. David, J. Gershenzon and W.W. Weisser corrected and commented on the manuscript.

Manuscript VI

Entomopathogens induce the transgenerational wing induction in pea aphids, *Acyrthosiphon pisum* (Homoptera: Aphididae)
Hatano E., Baverstock J., Pell J., Kunert G., Weisser W.W. In preparation for publication in the *Journal of Invertebrate Pathology*.

This manuscript addresses the second question, exploring whether specialist and generalist entomopathogens also induce wing formation by the same mechanism triggered by predators and parasitoids. Pea aphids that are infected by either *Pandora neoaphidis* (a specialist aphid pathogen) or *Beauveria bassiana* (a generalist entomopathogen) produce a higher proportion of winged morphs than do uninfected aphids. The results, however, indicate that the mechanism is different from that triggered by arthropods' natural enemies.

E. Hatano conducted the experiment, analysed the data and wrote the manuscript. All authors participated in the design of this experiment. J. Baverstock and J.K. Pell assisted in the experiment. All coauthors corrected and commented on the manuscript.

Aphid wing induction and ecological costs of alarm pheromone emission under field conditions

Eduardo Hatano[1], Grit Kunert[2], Wolfgang W. Weisser[1]

[1]Institute of Ecology, Friedrich Schiller University
[2]Biochemistry Department, Max Planck Institute for Chemical Ecology

Accepted in PLoS ONE in January 2010

ABSTRACT

The pea aphid, *Acyrthosiphon pisum* Harris, (Homoptera: Aphididae) releases the volatile sesquiterpene (*E*)-β-farnesene (EBF) when attacked by a predator, triggering escape responses in the aphid colony. Recently, it was shown that this alarm pheromone also mediates the production of the winged dispersal morph under laboratory conditions. The present work tested the wing-inducing effect of EBF under field conditions. Aphid colonies were exposed to two treatments (control and EBF) and tested in two different environmental conditions (field and laboratory). As in previous experiments aphids produced higher proportion of winged morphs among their offspring when exposed to EBF in the laboratory but even under field conditions the proportion of winged offspring was higher after EBF application (6.84±0.98%) compared to the hexane control (1.54±0.25%). In the field the proportion of aphids that survived in the test was lower in the EBF treatment (58.09±5.50%) than in the control (66.90±4.59%), in contrast to the identical test in the climate chamber in which most aphids survived in both treatments. Our results show that the role of EBF in aphid wing induction is also apparent under field conditions and they may indicate a potential cost of EBF emission, and emphasizes the importance of investigating the ecological role of induced defences under field conditions.

INTRODUCTION

Aphids are important economic insects in temperate regions, damaging plants by sucking nutrients from the phloem and transmitting plant viruses [1,2]. Because of their abundance, aphids are attacked by a wide range of predators such as ladybirds, lacewings and hoverfly larvae, all of which showed to influence strongly the growth and persistence of aphid colonies [3].

In response to a predator direct attack, aphids secrete cornicle droplets from a pair of tube-like structures on the abdomen called siphunculi [4-6]. The droplets glue together the predator's mouthparts [4], and in addition, they contain an alarm pheromone, the sesquiterpene (*E*)-β-farnesene (EBF), which is for some aphid species the main or only pheromone compound present [6-11]. EBF triggers various behavioural reactions in aphids, like withdrawing the stylets from the plant, or dropping off their host plants [12,13]. EBF may also attract some species of aphid predators [14-16] and parasitoids [17] and might be used by plants to deter aphids [18].

Polyphenism is one of the main characters of aphids and during the phase of asexual production in summer, both winged and unwinged females occur. In the case of the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), wing formation among offspring is maternally induced when the mother is under adverse biotic conditions, for example, triggered by crowding, low host plant quality, or the presence of natural enemies [19-28]. Recently, EBF was also found to mediate indirectly the production of winged offspring of the pea aphid [29], by increasing the number of tactile stimuli among individuals of a colony (pseudo crowding effect) [23,29]. This effect is analogous to the response of aphids to an increasing colony size (crowding), when the number of tactile interactions also increases [26]. While predator-induced

wing formation in pea aphids [19,20,24,28,30] and its mediation by EBF [29] were repeatedly demonstrated in the laboratory, the importance under natural conditions has so far not been investigated. It is conceivable that air movements change the amount and/or concentration of detectable EBF in an aphid colony, possibly alerting fewer aphids than under laboratory conditions. In addition, many pea aphids that perceive EBF walk away from the original plant and often do not survive during migration because of starvation or ground predators [31]. Both effects decrease the population density on the plant and, consequently, may weaken the pseudo-crowding effect and the production of winged morphs. Furthermore, the aphid alarm pheromone can act as kairomone by attracting natural enemies [32], and predation would further lower the number of aphids in a colony and also reduce the pseudo crowding effect [20].

In the current study, we tested the hypothesis that pea aphids under field conditions also produce higher proportion of winged offspring after reacting to EBF like observed in laboratory experiments. Our objectives were to determine: (I) to investigate the role of EBF for wing induction under field conditions and to compare it to a laboratory test, (II) to analyse the effect of EBF on aphid survival and fecundity. We exposed colonies of pea aphids daily to the alarm pheromone under field and laboratory conditions, scored the proportion of winged offspring and the number of individuals on the plants at the end of experiment. The alarm pheromone induced wing formation in offspring under field conditions confirming the transgenerational effect of predation risk for aphid dispersal. The alarm signalling also had a strong influence in the remaining proportion of aphids.

MATERIALS AND METHODS

Plant and aphid material

Pink pea aphids of clone BP [29] were reared on 3-week-old broad bean plants, *Vicia faba* L (variety The Sutton; Nickerson-Zwaan, UK). Plants were cultured in pots (10 cm diameter, 8 cm high) and covered with air-permeable bags (L x W = 39 x 20 cm, Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) to avoid aphid escape. Infested plants were kept in the climate chamber under constant conditions (16:8 L:D; 20^{o}C; 75% RH).

Aphid lines

Twenty-eight aphid lines were set up as described by Kunert *et al.* [29]. One aphid line consisted of the genetically identical progeny of a single aphid. For one line, one adult aphid was first placed on a three-week-old broad bean plant, allowed to reproduce for 48 hr, and then removed from the plant. After nine days, the daughters (10 aphids per line), now adults, were transferred to five new plants (two aphids per plant) to avoid crowding. After 48 hr reproducing, the daughters were removed, leaving 12 granddaughters per plant. After another six days, the granddaughters became third- and fourth-instar nymphs and 60 aphids from each line were transferred to four new broad bean plants in groups of 15 aphids. The four plants per line were

randomly allocated to one of four treatments (see below). In this way, both maternal effects and any effects of the plants on which aphids were reared were distributed equally over all treatments.

Experimental design

We tested the effect of EBF on aphid wing induction by exposing aphid colonies to either artificial EBF (EBF treatment) or a solvent control (control treatment) three times per day along five days. The experiment was set up simultaneously in two locations: in the field and in the climate chamber, resulting in a 2 (pheromone application) x 2 (location) factorial design.

Field test

Pairs of plants with aphids (granddaughters) from the same line were placed at a distance of five metres from one another and 10 m between pairs along the margins of the Jena Biodiversity field experiment [33] in Jena. The daily means of temperature ranged from 17.4^{o}C to 20.3^{o}C, relative humidity ranged from 75.9% to 88.2%, precipitation ranged from 0.007 mm to 0.566 mm, and wind speed ranged from 0.8 m/s to 21.2 m/s over the 5-day experimental period. One of the plants of a pair was allocated to the EBF treatment, the other one to the control. A toothpick holding a square piece (1 x 1 cm) of filter paper was fixed inside each pot in the soil. To reduce the access of natural enemies to aphid colonies, all plants were enclosed by cages, 30 cm in height, made from aluminium mesh (mesh width, 2mm) fixed using adhesive to a plastic frame of a plant saucer (25 cm i.d.) from which the bottom was removed. Cages were sprayed with insect glue (Soveurode, Witasek) and the bottom edges were pressed into the ground and covered with soil to prevent contact with other organisms.

For five days, 5 µl of EBF solution containing 1000ng EBF (0.20 µg EBF per 1 µl hexane; EBF treatment) or 5 µl hexane (control) were applied three times a day (at 8:00, 13:00 and 18:00) onto the filter paper of each pot through the mesh of the cages using a micropipette. The amount of EBF applied was enough to be perceived by the aphids and to elicit the alarm behaviour under field conditions. In addition, this amount was also used by Kunert *et al.* [29] who discernibly showed that the frequency of EBF emission per day rather than amount of EBF emitted regulates the proportion of wing offspring produced.

After five days, the adult aphids on the broad bean plants were counted and removed. Plants with aphids were covered with cellophane bags and transferred to the climate chamber with same conditions described above and kept until all nymphs became L4/adults. When offspring had reached maturity, all aphids from each plant were removed from the plant and frozen at -18°C, after which offspring number and offspring phenotype were counted.

Climate chamber test

The second pair of infested broad bean plants from each line was kept under climate chamber conditions (16:8 L:D; 20^{o}C; 75% RH) as a positive control. Plants were covered with cellophane bags so aphids could not escape. EBF was applied and aphids were handled exactly as in the field.

Statistical Analysis

All analyses were carried out with the R software version 2.8.1. The survival of adult aphids and the number of offspring produced were analysed using generalized linear models (GLM). Because overdispersion was detected during analysis, a quasibinomial (for proportion of aphid survival) and quasipoisson (for offspring count data) error structures were used in our analyses [34,35]. Because non-normality of the data proportion of winged morphs were square root transformed and analysed by an ANCOVA, using the total number of offspring as a covariate. In all models, aphid lines were included as a random effect. Models were simplified by reducing non-significant interactions followed by independent variables that were not included in any significant interaction [36]. Among non-significant independent variables or interactions with same number of variables, the one with highest p value was first removed followed by others in a descending order. After removing a non-significant interaction or variable, a new model was generated and only accepted if the removal did not significantly increase deviance comparing to the previous model after a F test (P>0.05) [37]. Otherwise, the previous model was retained and the simplification continued with the next non-significant interaction or variable. When an interaction of variables was found significant, the corresponding levels were compared using contrasts [36]. Results are presented as mean $\pm$ SE.

RESULTS

Survival of adult aphids

One replicate of the field control treatment was removed after the first day of experiment because all aphids from this plant disappeared. In the laboratory, proportion of adult aphids (granddaughters) that were found on the plants at the end of experiment was very high (97.02 $\pm$ 0.72%) regardless of the pheromone treatment, i.e. on average less than one aphid died over the five-day experimental period. In contrast, survival was much lower in the field where on average less than a third of the 15 aphids survived (27.27 $\pm$ 2.68%, $t_{108} = 13.939$, $p < 0.001$, Fig. 1A).

The application of alarm pheromone resulted in a significant lower proportion of adult aphids remaining on the plant (58.09 $\pm$ 5.50%) compared to the control (66.90 $\pm$ 4.59%, $t_{108} = 3.331$, $p < 0.01$, Fig. 1B). Although there was no interaction between the experiment location and pheromone treatment ($F_{1,108} = 2.22$, $p = 0.13$), we compared the survival of mothers in the EBF treatment and control separately for both locations to investigate the possible negative effects of EBF for aphids. For this purpose, we performed the same GLM test with a quasibinomial error distribution using orthogonal contrasts [38]. In the climate chamber, the number of remaining adult aphids did not differ between control and EBF treatment ($t_{55} = 8.144$, $p = 0.766$). However, in the field, survival of adult aphids in the EBF treatment was significantly lower and only 55% of that of aphids in the control ($t_{53} = 4.134$, $p < 0.001$). Although cages protected the plants from natural enemies, some ants were observed in few cages at the end of experiment which could also have influenced the survival of adults.

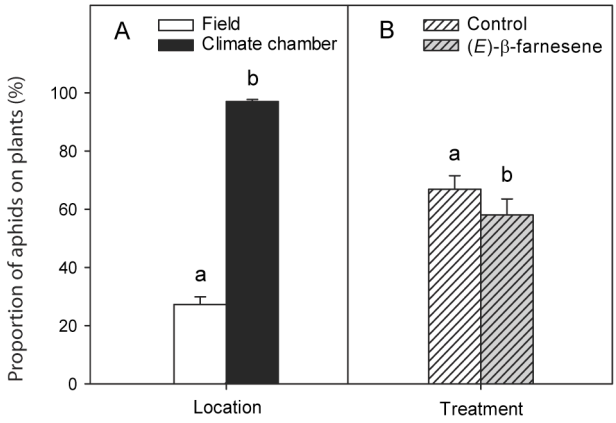

Figure 1. Proportion of remaining adult pea aphids exposed to A) alarm pheromone and control (left), and B) under field and climate chamber conditions (right). Initially, 15 aphids were introduced to each plant and the proportions of remaining adult aphids were recorded after five days in the field (open bar) and in the climate chamber (full bar) (t_{108} = 13.939, $p < 0.001$), and treated with (*E*)-β-farnesene (hatched full bar) and control (hatched open bar) treatments (t_{108} = 3.331, $p < 0.01$). Bars with different letters are statistically significant different ($p < 0.001$) within each group. The bars show mean values + SE.

Total number of offspring

In total, 28273 offspring were counted in the experiment. Significantly more offspring were recorded in the climate chamber than in the field (t_{107} = 10.102, $p < 0.001$), and more offspring were born in the control than in the EBF treatment (t_{107} = 4.414; $p < 0.001$). The interaction between location and pheromone application was significant ($F_{1,107}$ = 11.969, $p < 0.001$), i.e. the difference between control and EBF treatment was dependent on the location: a significant difference between EBF and control under field conditions (t_{53} = 76.862, $p < 0.001$; Fig. 2) but not under climate chamber conditions (t_{55} = 0.750, $p = 0.455$; Fig. 2).

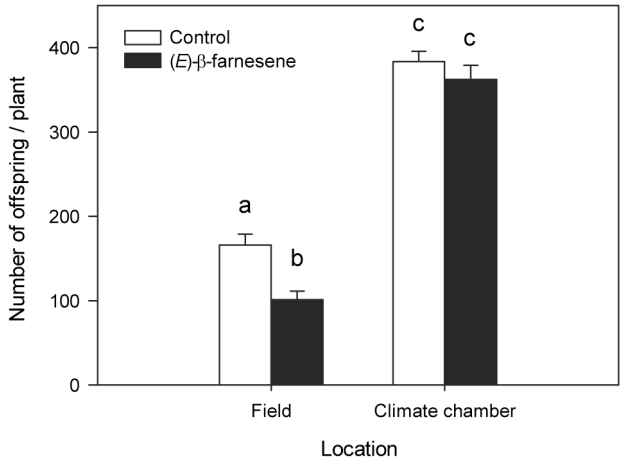

Figure 2. Colony sizes of aphids exposed to alarm pheromone and control under different conditions. Offspring on each plant were counted after five days of experiment in the field and in the climate chamber, when aphid colonies were treated with either (*E*)-β-farnesene (black bars) or control (white

bars) ($F_{1,107}$ = 11.969, p < 0.001). Bars with different letters are statistically significant different (p < 0.001). The bars show mean values + SE.

Offspring phenotype

Whilst the proportion of winged morphs among the offspring was higher in the climate chamber compared to the field (t_{103} = 1.113; p < 0.001), the application of EBF significantly increased wing induction (t_{103} = 1.138; p < 0.001, Fig. 3). The interaction between location and pheromone application was also significant ($F_{1,103}$ = 38.784, p < 0.001, Fig. 3). In the climate chamber, the proportion of winged morphs among the offspring was on average 124% higher in the EBF treatment than in the control (t_{55} = 10.444, p < 0.001, Fig. 3). In the field, the proportion of winged offspring increased by 600% from the control to the EBF treatment (t_{54} = 2.786, p < 0.01, Fig. 3).

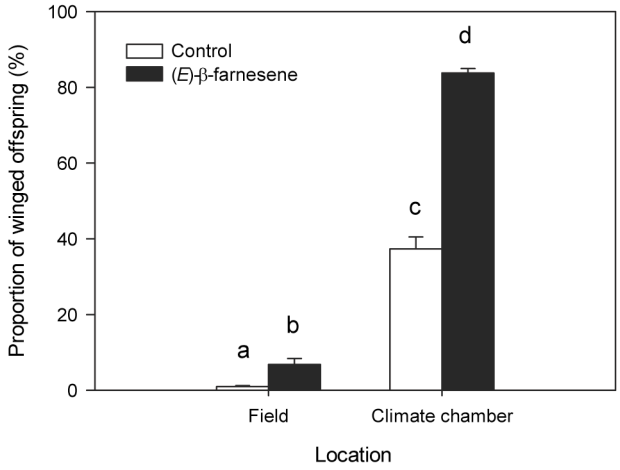

Figure 3. Induction of wing formation in offspring from colonies exposed to alarm pheromone and control under different conditions. The proportions of winged morphs among offspring were recorded in the field and in the climate chamber, for both the (*E*)-β-farnesene (black bars) and control (white bars) treatments ($F_{1,103}$ = 38.784, p < 0.001). Bars with different letters are statistically significant different (p < 0.01). The bars show mean values + SE.

The interaction among location, pheromone application and number of offspring was also significant ($F_{2,103}$ = 13.788; p < 0.001, Fig. 4): in the field, the proportion of winged offspring was not correlated to the number of offspring in the control treatment (0.2679 + 0.0025X, R^2 = 0.058, $F_{1,25}$ = 1.525, p = 0.228, Fig. 4), while there was a positive correlation in the EBF treatment (-0.420 + 0.019X, R^2 = 0.448, $F_{1,26}$ = 21.08, p < 0.01, Fig. 4). Under climate chamber conditions, the opposite was observed: the number of offspring positively affected the proportion of winged morphs in the control (1.920 + 0.011X, R^2 = 0.209, $F_{1,26}$ = 7.146, p= 0.0126, Fig. 4), but did not in the EBF treatment (9.306 - 0.00041X, R^2 = 0.011, $F_{1,26}$ = 0.288, p = 0.596, Fig. 4).

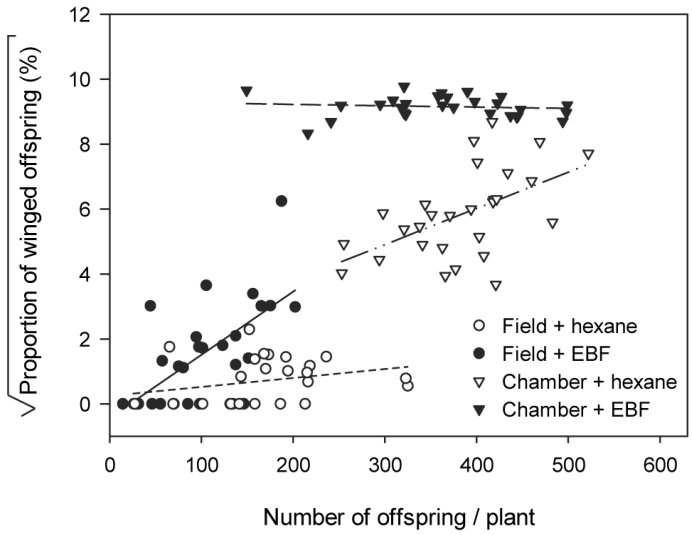

Figure 4. Wing induction of offspring in different colony sizes exposed to alarm pheromone and control under different conditions. The square root transformed proportion of winged offspring as a function of the number of offspring in the field and in the climate chamber, treated with either EBF or hexane. Open circles represent field colonies treated with hexane control (0.2679 + 0.0025X, R^2 = 0.058, $F_{1,25}$ = 1.525, p = 0.228); black circles are field colonies treated with EBF (-0.420 + 0.019X, R^2 = 0.448, $F_{1,26}$ = 21.08, p < 0.01); open triangles are chamber colonies treated with hexane control (1.920 + 0.011X, R^2 = 0.209, $F_{1,26}$ = 7.146, p = 0.0126); and black triangles are chamber colonies treated with EBF (9.306 - 0.00041X, R^2 = 0.011, $F_{1,26}$ = 0.288, p = 0.596).

DISCUSSION

While laboratory experiments are an important tool in revealing ecological mechanisms, field experiments are needed to test the ecological relevance of the observed effects. Our results show for the first time that EBF mediates the production of winged pea aphid offspring along with colony size under field conditions. In addition, our experiment showed that the proportion of adult remaining on plants was not only lower in the field than in the climate chamber (Fig. 1A), but it was also negatively affected by the application of EBF (Fig. 1B), resulting in fewer offspring than in the hexane control (Fig. 2).

Pea aphids trigger the production of winged morphs when in repeated physical contact with each other, as in the case of high aphid densities on a plant, which indicates high intraspecific competition levels (crowding effects; [26]). Therefore, smaller colonies are less likely to produce winged morphs than larger ones because of less physical contact between colony members [26]. Yet the proportion of dispersal morphs was higher in the EBF treatment, even though only 2.9 $\pm$ 0.5 adults remained on the EBF treated plant compared to plants treated with hexane in which 5.2 $\pm$ 0.6 adults remained (Fig. 1 and 3). When aphid colonies are exposed to EBF in laboratory conditions, the proportion of winged offspring increased with the initial number of aphids on a plant [39]. The climate chamber data reported here are very similar to those of the aphid group size of 13 in Kunert *et al.* [39]. In contrast, Kunert *et al.* [39] reported winged offspring production of 10% (control) and ca. 40% (EBF treatment) when initial aphid number was two. Although the number of remaining adult aphids in our field test was higher than two, the percentage of winged

offspring observed was lower. This indicates that wing induction in the field is reduced not only by lower number of mothers (Fig. 1) but also by other factors. Airflow in the field very likely quickly reduces the amount and concentration of EBF that reaches aphids, such that possibly only aphids near the source perceive biologically relevant amounts of EBF, resulting in a general decrease in the response. The increase of produced offspring enforced the pseudo crowding and crowding effects on each plant when EBF or control hexane was applied, respectively, and, therefore, also played a positive role in wing induction (cf. [20], Fig. 4). In addition, the large cages in the field test allow aphids to walk off the host plant and this might reduce the contact rate among individuals compared to the smaller cellophane bags in the climate chamber, where aphids leaving the plant are likely to return to it immediately. Finally, while the same aphid clone and the same plant species was used in the present experiment and in the experiments of Kunert *et al.* [29], small differences in manipulation may also have influenced the response of the experimental aphids towards the wing-inducing cues.

In the laboratory, there was no effect of EBF on adult aphid survival, indicating that the concentrations of EBF or hexane applied were both not toxic to the pea aphids nor led to a significant reduction in survival of individuals that left the plant upon application of the pheromone. Both, experiment location and application of solutions, independently affected the proportion of aphids that were found on plants at the end of the experiment. While aphids enclosed in cellophane bags could not move away far from their plants and were therefore likely to find the plant again after leaving it, aphids in larger field cages were likely to spend more time searching for their hosts, increasing the possibility for desiccation or starvation and thus decrease in fecundity [40-42]. A significant reduction in the number of adults on plants treated with EBF was also made by Wohlers [31] who reported that when pea aphids were dislodged by exposure to synthetic EBF they moved towards neighbouring plant models while a small proportion of aphids climbed back to the original plant. By making use of the alarm signalling behaviour, Bruce *et al.* [43] successfully reduced the aphid population in field plots using plant extracts containing 70% EBF and a slow-release point sources which probably resembled the natural emission of EBF from aphids [44]. An additional cost of the alarm pheromone perception might be the higher predation risk of aphids which left the plant [45]. Although the plants in the field were protected with cages, ants were able to enter the cages from below; hence it is likely that not only starvation but also predation contributed to the observed decrease the numbers of aphids in the field. A relationship between aphid alarm pheromone and ant aggression was reported before. In a comprehensive study, Nault *et al.* [46] exposed several myrmecophilous and non-myrmecophilous aphid species in a laboratory setting to ants, predators and alarm pheromone. Ants near myrmecophilic aphids became very aggressive in the presence of EBF and increased their rate of attack on aphid predators, but they did not attack aphids. However, when an alarm pheromone was applied to colonies of untended aphid species, ants became aggressive towards the aphids and sometimes carried them off the plant [46]. Similar observations of aggressive behaviour of aphid-attending ants towards an EBF source were made in the field [47,48].

Costs of alarm signalling was recently discussed by Verheggen *et al.* [49], who demonstrated that pea aphids regulate the emission of EBF according to social environment, with small colonies releasing less EBF than large colonies. In this context, aphids reduce the predation risk by not

attracting natural enemies and remaining inconspicuous while they reduce physiological cost to produce EBF.

In conclusion, our study shows that EBF mediates wing induction in pea aphid colonies not only under laboratory but also under natural conditions. The experiment under natural conditions also pointed to the importance of colony size in interaction with alarm signalling to produce winged offspring by the pseudo crowding effect. Now since we know that wing induction in aphids also occur under natural conditions it is important to investigate whether there is an ecological cost involved in alarm pheromone emission in detail.

ACKNOWLEDGEMENTS

We thank Dr. Sybille Unsicker for building the field cages, Dr. John Sloggett for his valuable comments on this manuscript, Ingrid Jakobi for helping rearing the plants, and for the comments of two anonymous reviewers.

REFERENCES

1. Katis NI, Tsitsipis JA, Stevens M, Powell G (2007) Transmission of plant viruses. In: Emden HFv, Harrington R, editors. Aphids as crop pests. Wallingford: CABI. pp. 353-390.
2. Quisenberry SS, Ni X (2007) Feeding injury. In: van Emden HF, Harrington R, editors. Aphids as crop pests. Wallingford: CABI. pp. 331-352.
3. Dixon AFG (1998) Aphid ecology. London: Chapman & Hall. 300 p.
4. Dixon AFG (1958) The escape responses shown by certain aphids to the presence of the coccinelid *Adalia decempunctata* (L.). Trans R Ent Soc London 110: 319-334.
5. Foldi-Hope C (1990) Ultrastructure of the corniculi of *Rhopalosiphum padi* L (Homoptera, Aphididae). Int J Insect Morphol Embryol 19: 25-33.
6. Kislow CJ, Edwards LJ (1972) Repellent odor in aphids. Nature 235: 108-109.
7. Bowers WS, Nault LR, Webb RE, Dutky SR (1972) Aphid alarm pheromone: isolation, identification, synthesis. Science 177: 1121-1122.
8. Edwards LJ, Siddall JB, Dunham LL, Uden P, Kislow CJ (1973) Trans-beta-farnesene, alarm pheromone of the green peach aphid, *Myzus persicae* (Sulzer). Nature 241: 126-127.
9. Francis F, Martin T, Lognay G, Haubruge E (2005) Role of (*E*)-beta-farnesene in systematic aphid prey location by *Episyrphus balteatus l*arvae (Diptera: Syrphidae). Eur J Entomol 102: 431-436.
10. Nault LR, Edwards LJ, Styer WE (1973) Aphid alarm pheromones: secretion and reception. Environ Entomol 2: 101-105.
11. Pickett JA, Griffiths DC (1980) Composition of aphid alarm pheromones. J Chem Ecol 6: 349-359.
12. Montgomery ME, Nault LR (1977) Comparative response of aphids to the alarm pheromone, (*E*)-beta-farnesene. Entomol Exp Appl 22: 236-242.
13. Wohlers P (1980) Escape responses of pea aphids, *Acyrthosiphon pisum* to alarm pheromones and additional stimuli. Entomol Exp Appl 27: 156-168.
14. Acar EB, Medina JC, Lee ML, Booth GM (2001) Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). Can Entomol 133: 389-397.
15. Al Abassi S, Birkett MA, Pettersson J, Pickett JA, Wadhams LJ, *et al.* (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. J Chem Ecol 26: 1765-1771.
16. Boo KS, Chung IB, Han KS, Pickett JA, Wadhams LJ (1998) Response of the lacewing *Chrysopa cognata* to pheromones of its aphid prey. J Chem Ecol 24: 631-643.

17. Micha SG, Wyss U (1996) Aphid alarm pheromone (*E*)-ß-farnesene: a host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). Chemoecology 7: 132-139.
18. Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, *et al.* (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. P Natl Acad Sci USA 103: 10509-10513.
19. Dixon AFG, Agarwala BK (1999) Ladybird-induced life-history changes in aphids. Proc R Soc Lond Ser B-Biol Sci 266: 1549-1553.
20. Kunert G, Weisser WW (2003) The interplay between density- and trait-mediated effects in predator-prey interactions: a case study in aphid wing polymorphism. Oecologia 135: 304-312.
21. Lees AD (1966) Photoperiodic timing mechanisms in insects. Nature 210: 986-989.
22. Müller CB, Williams IS, Hardie J (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecol Entomol 26: 330-340.
23. Sloggett JJ, Weisser WW (2004) A general mechanism for predator- and parasitoid-induced dispersal in the pea aphid, *Acyrthosiphon pisum*. Aphids in a New Millennium. pp. 79-85.
24. Sloggett JJ, Weisser WW (2002) Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*. Oikos 98: 323-333.
25. Sutherland ORW (1970) An intrinsic factor influencing alate production by two strains of pea aphid, *Acyrthosiphon pisum*. J Insect Physiol 16: 1349-1354.
26. Sutherland ORW (1969) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. J Insect Physiol 15: 1385-1410.
27. Sutherland ORW (1967) Role of host plant in production of winged forms by a green strain of pea aphid *Acyrthosiphon pisum* Harris. Nature 216: 387-388.
28. Weisser WW, Braendle C, Minoretti N (1999) Predator-induced morphological shift in the pea aphid. P Roy Soc B-Biol Sci 266: 1175-1181.
29. Kunert G, Otto S, Rose USR, Gershenzon J, Weisser WW (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecol Lett 8: 596-603.
30. Podjasek JO, Bosnjak LM, Brooker DJ, Mondor EB (2005) Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*. Can J Zoolog 83: 1138-1141.
31. Wohlers P (1981) Effects of the alarm pheromone (*E*)-beta-farnesene on dispersal behavior of the pea aphid *Acyrthosiphon pisum*. Entomol Exp Appl 29: 117-124.
32. Hatano E, Kunert G, Bartram S, Boland W, Gershenzon J, *et al.* (2008) Do aphid colonies amplify their emission of alarm pheromone? J Chem Ecol 34: 1149-1152.
33. Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, *et al.* (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl Ecol 5: 107-121.
34. Crawley MJ (2007) Count data. In: Crawley MJ, editor. The R book. Chichester: John Wiley & Sons Ltd. pp. 527-548.
35. Crawley MJ (2007) Proportion data. In: Crawley MJ, editor. The R book. Chichester: John Wiley & Sons Ltd. pp. 569-626.
36. Crawley MJ (2002) Statistical computing. West Sussex: John Wiley & Sons. 761 p.
37. Crawley MJ (2007) Generalized linear models. In: Crawley MJ, editor. The R book. Chichester: John Wiley & Sons Ltd. pp. 511-526.
38. Crawley MJ (2007) Statistical modelling. In: Crawley MJ, editor. The R book. Chichester, England: John Wiley & Sons Ltd. pp. 323-386.
39. Kunert G, Trautsch J, Weisser WW (2007) Density dependence of the alarm pheromone effect in pea aphids, *Acyrthosiphon pisum* (Sternorrhyncha : Aphididae). Eur J Entomol 104: 47-50.
40. Dill LM, Fraser AHG, Roitberg BD (1990) The economics of escape behavior in the pea aphid, *Acrythosiphon pisum*. Oecologia 83: 473-478.
41. Losey JE, Denno RF (1998) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecol Entomol 23: 53-61.
42. Roitberg BD, Myers JH (1978) Adaptation of alarm pheromone responses of pea aphid *Acyrthosiphon pisum* (Harris). Can J Zoolog 56: 103-108.
43. Bruce TJA, Birkett MA, Blande J, Hooper AM, Martin JL, *et al.* (2005) Response of economically important aphids to components of *Hemizygia petiolata* essential oil. Pest Manag Sci 61: 1115-1121.
44. Schwartzberg EG, Kunert G, Stephan C, David A, Rose USR, *et al.* (2008) Real-time analysis of alarm pheromone emission by the pea aphid (*Acyrthosiphon pisum*) under predation. J Chem Ecol 34: 76-81.
45. Roitberg BD, Myers JH, Frazer BD (1979) Influence of predators on the movement of apterous pea aphids between plants. J Anim Ecol 48: 111-122.

46. Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association - Role of aphid alarm pheromone. Science 192: 1349-1351.
47. Mondor E, Addicott J (2007) Do exaptations facilitate mutualistic associations between invasive and native species? Biol Invasions 9: 623-628.
48. Xiangyu JG, Zhang F, Fang YL, Kan W, Zhang GX, *et al.* (2002) Behavioural response of aphids to the alarm pheromone component (E)-beta-farnesene in the field. Physiol Entomol 27: 307-311.
49. Verheggen FJ, Haubruge E, De Moraes CM, Mescher MC (2009) Social enviroment influences aphid production of alarm pheromone. Behav Ecol 20: 283-288.

Chemical cues mediating aphid location by natural enemies

Eduardo Hatano[1], Grit Kunert[2], John Paul Michaud[3], Wolfgang W. Weisser[1]

[1]Institute of Ecology, Friedrich Schiller University
[2]Biochemistry Department, Max Planck Institute for Chemical Ecology
[3]Agricultural Research Center, Kansas State University

Published in the *European Journal of Entomology* 105, 797-806 (2008).

REVIEW

Eur. J. Entomol. **105**: 797–806, 2008
http://www.eje.cz/scripts/viewabstract.php?abstract=1400
ISSN 1210-5759 (print), 1802-8829 (online)

Chemical cues mediating aphid location by natural enemies

EDUARDO HATANO[1, 2], GRIT KUNERT[2], J.P. MICHAUD[3] and WOLFGANG W. WEISSER[1]

[1]Institute of Ecology, Friedrich-Schiller-University Jena, Dornburger Str. 159, 07745 Jena, Germany;
e-mails: ehatano@ice.mpg.de, wolfgang.weisser@uni-jena.de

[2]Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany; e-mail: gkunert@ice.mpg.de

[3]Kansas State University, Agricultural Research Center-Hays, 1232 240th Ave., Hays, KS 67601, USA; e-mail: jpmi@ksu.edu

Key words. Aphid, natural enemies, host selection, semiochemicals, infochemical

Abstract. There is increasing evidence that chemical cues play a pivotal role in host selection by the natural enemies of aphids. We use Vinson's (1976) division of the host selection process into habitat location, host location and host acceptance for both parasitoids and predators and review what is known about the role of semiochemicals in aphid selection by natural enemies. For habitat location (i.e. detection of the host plant), volatiles emitted by plants after aphid attack have been described for a number of plant-aphid interactions. These synomones indicate not only the presence of an aphid host plant to the predator or parasitoid, but also the presence of aphids. Volatiles emitted from undamaged host plants are often attractive to aphid parasitoids, but less so for predators. Host location by the natural enemy on the food plant is guided by semiochemicals that mostly originate from the aphids, in particular aphid alarm pheromone, honeydew, or the smell of the aphid itself. Host acceptance is guided by contact chemicals for both predators and parasitoids. In parasitoids, host recognition may be based on visual cues or on contact chemicals on the aphid's cuticle, whereas host acceptance is ultimately based on as yet unknown substances within the aphid's hemolymph. While it appears that many predators and parasitoids are attracted to the same semiochemicals, synergistic and antagonistic interactions among chemical substances have only rarely been investigated. More research into model systems is needed, not only to identify important semiochemicals, but also to determine their range of attraction. Recent progress in the development of analytical techniques has created new opportunities to improve our understanding of the chemical ecology of aphid-natural enemy interactions in the coming years.

INTRODUCTION

Prey location in a complex environment, filled with different plants and animal species, is a complex task. Predatory and parasitic insects have specialized sensory nervous systems that allow them to use a variety of cues to find and identify target organisms. Cues can be physical such as colour, sound, shape and size as well as chemical and these may be useful for long or short range attraction to prey. In this paper, we focus on the chemical cues used by aphid predators and parasitoids to detect their aphid (Hemiptera: Aphididae) prey or host. In recent years, much insight has been gained into the chemical ecology of aphid-natural enemy interactions and the large number of articles describing new findings suggests that a review on the current state of our knowledge would be useful. Reviewing physical cues is beyond the scope of the current paper, although we refer to these cues wherever appropriate.

For parasitoids, Vinson (1976) divided the host selection process into three different steps. The first step is habitat location and the second is host location. We define habitat location as finding the host plant of an aphid species and host location as locating the aphid when the natural enemy is already on the plant. Thus, in our terminology, habitat location is analogous to food plant location. Habitat location may also be defined as finding the habitat of the host plant, e.g. a meadow. However, we believe that for aphid natural enemies the distinction between finding a plant and finding an aphid on the plant is more useful, even though it is likely that for some natural enemies the search for hosts may start before landing on the plant. Because aphids only occupy a fraction of all host plants available, first finding a plant and then searching for aphids may not be a very efficient strategy. For this reason, many aphid natural enemies do not search for a "host habitat" but for a "habitat with hosts".

In Vinson's (1976) terminology, the final step in the host selection process is host acceptance, which is the proper act of oviposition or host/prey consumption (cf. Steidle & van Loon, 2002). For parasitoids, this final step has been divided into host recognition and host acceptance (Michaud & Mackauer, 1994; Muratori et al., 2006). A host may be recognized visually, or by antennal contact with chemical cues in the aphid cuticle. Final host acceptance depends on an assessment of host quality made during ovipositor probing, but a host may be rejected either after recognition or after ovipositor probing.

Although Vinson (1976) developed his framework to characterize the parasitoid search process, it can also be used for aphid predators. Whereas in aphid parasitoids the host is primarily used for oviposition, it may also be used for host feeding, or as a source of honeydew. Aphid predators need to find aphids for both feeding and oviposition, unless they obtain food from non-aphid prey or plant sources. In general, host/prey selection by both

aphid predators and parasitoids is a step-wise process consistent with Vinson's framework.

We start with a brief discussion of the particular challenges involved in the use of chemical cues for host selection and then review what is known about the importance of chemical cues in each step of the host selection process. We will not review the signal cascades within the organisms (plants, aphids or natural enemies) that are involved in the production and detection of chemical cues. The responses of plants to feeding by aphids and other insect herbivores and the activation of signalling pathways have been recently reviewed (Walling, 2000; de Vos et al., 2007; Kempema et al., 2007).

THE DETECTABILITY-RELIABILITY PROBLEM AND THE SEARCH FOR CHEMICAL CUES

In an ecological context, *semiochemicals* are chemical substances that convey information between two organisms, evoking a behavioural or physiological response that is adaptive to one of the organisms or both (Vet & Dicke, 1992). Semiochemicals are classified, not with respect to their chemical properties or source, but rather according to their ecological roles (Nordlund & Lewis, 1976; Dicke & Sabelis, 1988; Vet & Dicke, 1992). While *pheromones* are semiochemicals released by one individual and perceived by another of the same species, *allelochemicals* are perceived by individuals of a different species. Allelochemicals can be further subdivided into different categories depending on the beneficiary of the signal. If the only beneficiary is the perceiver, the allelochemical is termed an *allomone*; if only the fitness of the producer is increased, it is termed a *kairomone*, and if both producer and perceiver benefit from the information exchange it is termed a *synomone*. The classification of a chemical compound may thus change according to the studied interaction. Pheromones of herbivores, for example, that may be perceived by a natural enemy and used to locate it are, for this interaction, classified as kairomones (Gabrys et al., 1997; Glinwood et al., 1999; Al Abassi et al., 2000).

On searching for herbivores, natural enemies face the dilemma of reliability versus detectability (Vet & Dicke, 1992). Volatiles produced directly by herbivores give reliable information about their presence, but occur in low concentrations in the environment due to the low biomass of aphids. Plant volatiles, on the other hand, are easily detected because of their high biomass, but are less reliable since plants may or may not harbor herbivores. Vet & Dicke (1992) suggested that, in general, natural enemies might use the following strategies to overcome the reliability-detectability problem: (1) use more conspicuous semiochemicals from herbivore stages other than the one susceptible to attack, (2) focus responses on stimuli created by specific interactions between the herbivore and its plant, or (3) learn to link easy-to-detect stimuli to reliable but hard-to-detect stimuli. The first possibility is not useful for aphid natural enemies as aphid stages have such low biomass, but the other two are frequently used.

SEMIOCHEMICALS INVOLVED IN THE HOST SELECTION PROCESS

Habitat location

Aphid natural enemies must first locate aphid habitat, i.e. a host plant where aphids might be present. Attraction to (undamaged) host plants has been shown for a number of aphid parasitoids (Hymenoptera: Braconidae), e.g. *Diaretiella rapae* (M'Intosh) (Read et al., 1970), *Lysiphlebus testaceipes* (Cresson) (Schuster & Starks, 1974), *Trioxys indicus* Subba Rao and Sharma (Singh & Sinha, 1982), *Aphidius uzbekistanicus* Luzhetski, *Aphidius ervi* Haliday (Powell & Zhang, 1983), *Aphidius rhopalosiphi* De Stephani-Perez, *Lysiphlebus fabarum* (Marshall), *Praon* sp. (Van Emden, 1988), and *Aphidius funebris* Mackauer (Pareja et al., 2007) so that at first sight it appears to be a general phenomenon in aphid parasitoids. However, the parasitoid *Aphidius nigripes* Ashmead is not attracted to the odour of potato plants, the host plant of the aphid *Macrosiphum euphorbiae* (Thomas) (Bouchard & Cloutier, 1985). The aphids *M. euphorbiae* and *Myzus persicae* (Sulzer) are the most common hosts of *A. nigripes*, but are very polyphagous species. Thus, it is possible that generalist parasitoids, or those that attack aphids with a wide host range, are less likely to respond to cues from plants without any additional evidence that aphids are present. However, *A. ervi* is also quite polyphagous, but is attracted by uninfested plants of its aphid hosts (Powell & Zhang, 1983), suggesting more study is needed before generalisations can be drawn.

In contrast to parasitoids, aphid predators mostly appear not to use odours of undamaged plants to locate aphids. While ladybirds and other aphid natural enemies frequently visit plants for purposes such as nectar or pollen feeding, and may be attracted to these plants in the absence of aphids (e.g. Michaud & Qureshi, 2005), laboratory studies have often failed to show attraction to undamaged plants. For example, the ladybird (Coleoptera: Coccinellidae) species *Coccinella septempunctata* L. (Ninkovic et al., 2001) and *Adalia bipunctata* (L.) (Francis et al., 2004), and the hoverfly *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) (Francis et al., 2005b) were not attracted to odours from their (undamaged) host plant. An exception is *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) that is attracted to plant volatiles (Hagen et al., 1976). The number of predator species tested is still low, but it appears that aphid predators, which are usually more polyphagous than parasitoids, are generally not attracted by the odour of undamaged plants without evidence of aphids. There is need for more testing, in particular for those species that not only feed on aphids but also feed on plant pollen, nectar and other plant parts.

The first evidence that plants can modify their volatile emissions in response to aphid attack was given by Guerrieri et al. (1993) who found that the parasitoid *A. ervi* was attracted to plants damaged by aphids, but not to undamaged plants. In fact, early evidence that damaged plants emit allelochemicals only after herbivory was given by Read et al. (1970) who showed attraction of

TABLE 1. List of chemical compounds from different aphid-plant complexes and their effect on natural enemies responses.

HABITAT LOCATION	Plant	Aphid	Natural enemy	Effect	Reference
6-methyl-5-hepten-2-one (MHO)	*V. faba*	*A. pisum*	*A. ervi*	Attract	Du et al., 1998; Powell et al., 1998
	C. nigra	*U. jacea*	*A. funebris*	Attract	Pareja et al., 2007
	C. nigra	*U. jacea*	*A. uzbekistanicus*	Repel	Holler et al., 1994
	T. avestivum	*R. padi*	*A. rhopalosiphi*	None	Gonzales et al., 1999
(*Z*)-3-hexenyl acetate	*C. nigra*	*U. jacea*	*A. funebris*	Attract	Pareja et al., 2007
Allyl isothiocyanate	*B. oleracea*	*B. brassicae*	*D. rapae*	Attract	Read et al., 1970
Methyl salicylate	*G. max*	*A. glycines*	*C. septempunctata*	Attract	Zhu & Park, 2005
			Syrphid flies	Attract	
(Z)-jasmone			*A. ervi*	Attract	Birkett et al., 2000
			C. septempunctata	Attract	
Benzaldehyde	*C. sinensis*	*T. aurantii*	*C. sinica*	Attract	Han & Chen, 2002a, b
			C. septempunctata	Attract	
HOST LOCATION					
Indole acetaldehyde	Lucerne		*Hippodamia* spp.	Attract	van Emden & Hagen, 1976
			C. carnea	Attract	
(*E*)-β-farnesene		*M. persicae*	*H. convergens*	Attract	Acar et al., 2001
			C. septempunctata	Attract	Al Abassi et al., 2000
		M. persicae *A. pisum*	*A. bipunctata*	Attract	Francis et al., 2004
		M. viciae *A. pisum* *A. fabae*	*E. balteatus*	Attract	Francis et al., 2005b
			P. melanarius	Attract	Kielty et al., 1996
			H. rufipes	Attract	
			N. brevicollis	None	Kielty et al., 1996
		S. avenae	*A. uzbekistanicus*	Attract	Micha & Wyss, 1996
			P. volucre	Attract	
		S. avenae	*L. testaceipes*	None	Micha & Wyss, 1996
			C. cognata	None	Boo et al., 1998
HABITAT AND HOST LOCATION					
(4a*S*,7*S*,7a*R*)-nepetalactone			*C. cognata*	Attract	Boo et al., 1998
			A. ervi	Attract	Glinwood et al., 1999
			A. eadyi	Attract	
(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol			*C. oculata*	Attract	Zhu et al., 2005
			P. volucre	Attract	Gabrys et al., 1997
			D. rapae	Attract	
HOST ACCEPTANCE					
long hydrocarbon chains, wax esters, alcohols, and aldehydes		*S. avenae*	*A. rhopalosiphi*	Accept	Muratori et al., 2006
		P. tessellatus	*F. tarquinius*	Accept	Lohman et al., 2006
		P. tessellatus	*C. slossonae*	Accept	Lohman et al., 2006
		P. tessellatus	*S. ribesii*	Accept	Lohman et al., 2006

Diaretiella rapae (M'Intosh) (Hymenoptera: Braconidae) to mustard oil, allyl isothiocyanate (Table 1), emitted by collard, *Brassica oleracea*, in response to damage by the aphid, *Brevicoryne brassicae* (L.) (Read et al., 1970). Thereafter, a number of studies reported attraction of both aphid parasitoids and predators to the "plant-aphid complex" (also "plant-host complex"). Because this term refers to a set-up where a natural enemy can choose between a plant where aphids have been feeding and a control, or clean plant, it is often not clear whether attraction is due to volatiles emitted by the plant, the aphid, or aphid residues on the plant. Guerrieri et al. (1993) tested plants where remains of aphids had been removed to show that the plant changed its volatile profile. A number of subsequent studies supported this result (Du et al., 1996, 1998; Guerrieri et al., 1999). For example, Du et al. (1996) used a no-choice wind-tunnel experiment to demonstrate that a higher percentage of naïve female parasitoids landed on broad beans, *Vicia faba* L., damaged by *Acyrthosiphon pisum* (Harris) than on undamaged plants or aphids alone. These experiments suggested that not only herbivore-induced plant volatiles can attract aphid parasitoids, but also that volatiles from exuviae and faeces do not influence parasitoid behaviour at long range, possibly because of their low detectability. It was clear from the results that the main stimuli attractive to parasitoids were released from the damaged plant and that

plants produce semiochemicals in concentrations sufficient to be detected by parasitoids.

Attraction to aphid-damaged plants has also been shown for predators such as *C. septempunctata and C. sinica* (Han & Chen, 2002a). Ninkovic et al. (2001) reported that *C. septempunctata* was attracted to odours from barley plants (*Hordeum volgare*) infested or previously infested by the aphid, *Rhopalosiphum padi* in a four-arm olfactometer assay, but not to volatiles from uninfested plants or undisturbed aphids. For both parasitoids and predators there is now increasing evidence for a role of plant-derived synomones in natural enemy habitat location.

The first study to identify aphid-induced volatiles was by Du et al. (1998), who tested the electroantennogram responses of *A. ervi* females to volatile compounds from broad bean, *V. faba*. Among the volatiles produced by the plant in response to aphid attack, 6-methyl-5-hepten-2-one (MHO) was identified to be mainly responsible for attraction of the parasitoid. A number of other compounds also elicited responses in an olfactometer, but only MHO elicited a parasitoid flight response (Powell et al., 1998). Since the seminal study by Du et al. (1998), a number of other model systems have demonstrated a special role for MHO (Table 1). It was even suggested that MHO acts as an elicitor of dispersal for the parasitoid *A. uzbekistanicus,* to avoid attack by the hyperparasitioid *Alloxysta victrix* (Westwood) (Hymenoptera: Alloxystidae), as MHO was detected in the headspace of the hyperparasitoid (Höller et al., 1994). MHO is, however, not attractive to all parasitoids. For example, MHO was detected among the entrained volatiles of wheat plants damaged by *R. padi*, but was not attractive to the parasitoid *A. rhopalosiphi* when offered alone (Gonzales et al., 1999). Interestingly, whereas MHO is induced in *V. faba* infested with *A. pisum* ostensibly to attract *A. ervi* females, *V. faba* infested with *Aphis fabae* Scopoli (Hymenoptera: Aphididae) does not produce this volatile chemical and attracts significantly fewer *A. ervi* females for which *A. fabae* is not a host (Du et al., 1996, 1998; Powell et al., 1998; Guerrieri et al., 1999). Clearly, the role of MHO needs to be investigated in more detail. Other compounds emitted from aphid-damaged plants that attract certain parasitoids are (Z)-3-hexenyl acetate and (Z)-jasmone (Table 1).

MHO appears to play no role in habitat location by aphid predators and other induced compounds have been implicated in guiding predator searching behaviour. Zhu & Park (2005) examined the volatile emissions of soybean plants, *Glycine max* L. and identified methyl salicylate (Table 1) as a compound induced by feeding of the aphid *Aphis glycines* Matsumura. When methyl salicylate was applied to the antennae of *C. septempunctata*, it elicited a positive EAG response and also attracted this predator and syrphid flies to traps (Zhu & Park, 2005). (*Z*)-jasmone (Table 1) is a volatile compound of plants known for activating plant defenses and will attract the predator *C. septempunctata* in a four-arm olfactometer and the parasitoid *A. ervi* in a wind tunnel (Birkett et al., 2000). Interestingly, (*Z*)-jasmone induced the emission of (*E*)-β-ocimene in *V. faba*, which also attracted the parasitoid (Birkett et al., 2000). Han & Chen (2002a, b) detected high amounts of benzaldehyde released from tea shoots, *Camellia sinensis* L., when damaged by *Toxoptera aurantii* (Boyer de Fonscolombe), which is probably formed from a cyanogenic glycoside hydrolyzed by salivary enzymes of the aphid (Table 1). Benzaldehyde elicited positive EAG responses and was attractive to *Aphidius* sp. parasitoids and the predators *Chrysopa sinica* Tjeder (Neuroptera: Chrysopidae) and *C. septempunctata* (Han & Chen 2002a, b). As in the case of parasitoids, the number of prey-predator systems investigated is increasing and it is likely that more compounds will be detected in the coming years.

One special case of habitat location semiochemicals are aphid sex pheromones. For many aphid species, sex pheromones were identified as a mixture of two monoterpenes, (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (-)-(1*R*,4a*S*,7*S*, 7a*R*)-nepetalactol (Table 1), in ratios that vary according to the species (Pickett et al., 1992). These volatile compounds have been shown to attract female parasitoids in the field (Hardie et al., 1991, 1994) and elicit oriented flights by *A. ervi* and *Aphidius eadyi* Starý females when they are added to a piece of filter paper next to *V. faba* plants in the lab (Glinwood et al., 1999). Other examples of attraction to aphid sex pheromones include the parasitoids *Praon volucre* (Haliday) (Hymenoptera: Aphidiidae) and *D. rapae* (Gabrys et al., 1997), and the predators *Chrysopa cognata* McLachlan (Neuroptera: Chrysopidae) (Boo et al., 1998), and *Chrysopa oculata* Say (Neuroptera: Chrysopidae) (Zhu et al., 2005). Although sex pheromones reliably indicate the presence of aphids, they are only useful foraging cues in autumn when sexual morphs are produced.

To summarize, the seminal study by Du et al. (1996) started the search for aphid-induced volatiles important for their location by predators and parasitoids. While such compounds are important for habitat location in many systems, many parasitoids are also attracted to host plants free of aphids. Further work is needed to (i) test attraction of predators to undamaged plants, (ii) identify volatile compounds from aphid-infested plants and (iii) test the range of attraction of these volatiles under field conditions.

Aphid location

Following habitat location, natural enemies use short range chemical and physical cues to search for a suitable herbivore on the host plant. Among physical cues, visual stimuli (colour and shape) and aphid movement have been shown to be important factors for a number of aphid parasitoids (e.g. Michaud & Mackauer, 1994, 1995) and predators (e.g. Harmon et al., 1998). For example, *A. ervi* prefers to oviposit into green rather than red clones of the aphid *A. pisum* (Michaud & Mackauer, 1994; Libbrecht et al., 2007). *Praon pequodorum* Viereck females rarely attack aphids when they are not moving (Michaud & Mackauer, 1995). The ladybirds (Coleoptera: Coccinellidae) *C. septempunctata*, *Hippodamia convergens* Guerin,

Harmonia axyridis (Pallas), and *Coleomegilla maculata* (DeGeer) selectively attack red and green clones of *A. pisum* (Harmon et al., 1998). Therefore, predators and parasitoids use a number of physical cues to locate aphids at short range and these may influence preferences for ovipositing or feeding on a particular species or clone.

Among the chemical cues used by natural enemies for aphid location, aphid honeydew was the first to be investigated (e.g. Bombosch & Volk, 1966). Initially considered to be an important kairomone for habitat location, studies with various parasitoids and predators have shown that it often acts as an arrestant, i.e. it increases the time that natural enemies search for aphids on plants. Often, the natural enemy needs physical contact with honeydew to change its behaviour (Dixon, 1959; Bombosch & Volk, 1966; Carter & Dixon, 1984; Budenberg, 1990; Budenberg & Powell, 1992; Budenberg et al., 1992; Ide et al., 2007). Aphid honeydew also acts as an oviposition stimulus for syrphid flies (Budenberg & Powell, 1992) and for the predatory gall midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (Choi et al., 2004). The spraying of artificial honeydew to increase numbers of *Hippodamia* spp. and *C. carnea* in lucerne fields in California is one well-known example of the use of kairomones in aphid control (Hagen et al., 1971). Predators respond positively to the odour of a breakdown product of tryptophane, probably indole acetaldehyde (Table 1, Van Emden & Hagen, 1976) and remain in the treated area even when aphids are absent. The usefulness of artificial honeydew in improving biological control has been repeatedly tested, for example to manipulate the spatial distributions of ladybirds (Ben Saad & Bishop, 1976; Evans & Richards, 1997). Hagen (1986) suggested that *C. carnea* detects a synonome from the crop before responding to the kairomone, but this has not yet been identified. Attraction to honeydew has been shown for the parasitoid *A. rhopalosiphi* (Gardner & Dixon, 1985; Hagvar & Hofsvang, 1989; Budenberg, 1990; Budenberg et al., 1992), although the range of detectability remains unclear. Recently, Choi et al. (2004) captured honeydew volatiles and found that the gall midge *A. aphidimyza* was attracted to them, suggesting that these may attract natural enemies from some distance. Results with the syrphid *E. balteatus* also suggest some attractant role of honeydew volatiles as females landed more frequently on corn ears contaminated with honeydew from *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) than on clean ears (Budenberg et al., 1992). However, the amount of volatiles released is probably small and unlikely to be effective as a long range attractant until plants become heavily infested.

In addition to aphid honeydew, the aphid alarm pheromone, (*E*)-β-farnesene (EBF, Table 1) can be an important kairomone in aphid location. It is commonly released in the cornicle secretions of many aphid species (Francis et al., 2005a) to alert surrounding aphids of the presence of natural enemies (Grasswitz & Paine, 1992; Francis et al., 2004, 2005b; Kunert et al., 2005). However, EBF is a sesquiterpenoid which reacts quickly with ozone (Pinto et al., 2007). Single aphids have peak emissions of < 50 ng (Schwartzberg et al., 2008) and usually only the attacked aphid emits, whereas undisturbed aphids in the vicinity do not (Hatano et al., 2008). Thus, concentrations of EBF may be undetectable at a distance from the plant, likely limiting its usefulness to short range attraction. EBF is attractive to a number of aphid natural enemies such as the parasitoids *A. uzbekistanicus* and *P. volucre* (Micha & Wyss, 1996), the syrphid *E. balteatus* (Francis et al., 2005b), the ladybirds *A. bipunctata* (Francis et al., 2004), *C. septempunctata* (Al Abassi et al., 2000), *Harmonia axyridis* Pallas (Harmel et al., 2007) and *H. convergens* (Acar et al., 2001), and the ground beetles *Pterostichus melanarius* Illiger (Coleoptera: Carabidae) and *Harpalus rufipes* (DeGeer) (Coleoptera: Carabidae) (Kielty et al., 1996). Presumably as a consequence of EBF emission, disturbed or squashed individuals of *A. pisum* and *M. persicae* attracted significantly higher numbers of *A. bipunctata* than undisturbed aphids (Francis et al., 2004) and the same was observed for the parasitoid *A. uzbekistanicus* attacking *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (Micha & Wyss, 1996). Interestingly, ladybird larvae can perceive EBF from an aphid captured by a conspecific larvae, alerting it to the presence of prey (Hemptinne et al., 2000). However, some aphid natural enemies are not attracted to EBF, including the predatory lacewing, *C. cognata*, and the parasitoid *L. testaceipes*, (Micha & Wyss, 1996; Boo et al., 1998). Francis et al. (2005a) analysed the volatiles emitted from squashed aphids and found 8 out of 23 species that emit other compounds in addition to EBF and two species that did not emit EBF. Thus, there are other volatiles emitted by aphids that are candidate compounds for host location.

It is important to point out that semiochemicals involved in host location do not necessarily lead the natural enemy directly to the aphid. Rather, substances indicating the presence of aphids may cue behaviours such as intensive local search which improve prey detection. For example, when perceiving aphid cues, or after capturing an aphid, ladybird larvae switch from an extensive search to an intensive, area-restricted search by increasing their frequency of turning and reducing their speed (Dixon, 1959, 2000).

To summarize, there is ample evidence for the involvement of chemical cues in host location but they remain poorly understood. The identity of the semiochemicals emitted by healthy or attacked aphids, or via honeydew, has not been established except for the aphid alarm pheromone, EBF, which appears to have broad activity. Detection distance appears to vary depending on the semiochemical and may also differ among species of predators and parasitoids.

HOST RECOGNITION AND ACCEPTANCE

Once an aphid is located, natural enemies have to recognize it as potential prey before they attack it. In addition, parasitoids use ovipositor probing to assess host quality before oviposition. For host recognition, chemical cues can be important, in particular contact kairomones

from the surface of aphid's cuticle. Most contact kairomones are not volatiles. Weinbrenner & Völkl (2002) showed that contact kairomones affect host recognition by the parasitoid *A. ervi*, since washed pea aphids were attacked less often than non-washed aphids. Shed exuviae of aphids often elicit oviposition responses by parasitoids e.g. *A. ervi* (Powell et al., 1998; Battaglia et al., 2000) and *A. rhopalosiphi* (Muratori et al., 2006).

Various isolation methods have been used to identify semiochemicals involved in host recognition. Han & Chen (2002a) used hexane and ether rinses of *T. aurantii* cuticles to test attraction of the parasitoid *Aphidius sp.*, and the predators, *C. sinica* and *C. septempunctata*. The hexane rinse was more attractive than the ether rinse, possibly because it contained benzaldehyde, but all cuticle rinses acted as short range cues or contact semiochemicals for all natural enemies tested. Chemical and physical characteristics of the exuviae of *S. avenae* were described by Muratori et al. (2006), who found a great variety of long hydrocarbon chains (C25 to C31), and a few wax esters, alcohols, and aldehydes (Table 1) in extractions. The extractions also elicited antennal contacts and attacks by *A. rhopalosiphi*, and heat treatment applied to destroy the structure of the epicuticular surface did not diminish responses, suggesting that wax structure *per se* does not determine host recognition. A similar role of hydrocarbons in aphid recognition was demonstrated for *Feniseca tarquinius* (Abbot) (Lepidoptera: Lycaenidae), *Chrysopa slossonae* Banks (Neuroptera: Chrysopidae), *Syrphus ribesii* (L.) (Diptera: Syrphidae), and for parasitoids of the genus *Lysiphlebus*, some of which chemically mimic the hydrocarbons of their prey to deceive aphid-tending ants (Völkl, 1992; Liepert & Dettner, 1993; Lohman et al., 2006).

Chemical and physical compounds in cornicle secretions are active at very short range or in direct contact with an aphid, usually stimulating attacks by parasitoids. This response to cornicle secretion appears to be innate and host specific (Battaglia et al., 1995, 2000). The parasitoid *L. testaceipes* responded differently toward *R. padi* homogenates and cornicle wax compared to those of the non-host *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae). However, when the non-host was covered with *R. padi*'s cornicle wax, *L. testaceipes* increased its attack frequency (Grasswitz & Paine, 1992). Cornicle secretion is only produced by aphids during attack (Goff & Nault, 1974) and can be an effective defensive weapon against predators and parasitoids. However, when the secretion is hardened it poses no threat to natural enemies and may serve as a kairomone for host recognition.

Parasitoids also perceive the internal chemistry of their hosts via receptors on the ovipositor. Thus, probing ("test stinging") of the host, although indicative of attack, is also an investigatory behaviour (Grasswitz & Paine, 1992; Powell et al., 1998). Le Ralec & Rabasse (1988) studied the ovipositors of Aphidiinae and described three pairs of valvulae, in which the third valvulae surround the other two. Mechano- and chemoreceptors are present on the first and/or second valvulae depending on the species (Larocca et al., 2007). The internal cues within aphids that elicit oviposition remain unknown, but it is clear that not all probes result in oviposition. Thus ovipositor probing is the final stage of host selection and terminates with acceptance or rejection. In species that use no visual cues such as *Ephedrus californicus* (Michaud & Mackauer, 1994), host acceptance is entirely a function of stimuli perceived during ovipositor probing.

Many aphid predators probably assess the quality of their prey using receptors on the maxillae only after attack and following contact with body fluids (Nakamuta, 1984; Nakamuta & Saito, 1985). Dixon (1958) reported that larvae of *A. decempunctata* only reject the poisonous *Hyalopterus pruni* (Geoffroy) (Hemiptera: Aphididae) after piercing its cuticle. Moreover, the same predator also fed on *A. fabae* and *M. viciae,* but regurgitated and released them after two minutes (Dixon, 1958).

Predators also use host cues to decide whether to lay eggs at a given site. For example, aphid odours and cornicle secretions induce oviposition in *C. septempunctata* (Evans & Dixon, 1986). However, residues left by predators in previous visits to plants, in particular "larval tracks" may deter ovipositions by adults of the same or other species. Initially this was shown for green lacewings, *Chrysopa oculata* Say (Neuroptera: Chrysopidae) (Růžička, 1994), and for ladybirds including *C. septempunctata* (Růžička, 1997). Subsequently, it has been shown for a number of other species such as *H. axyridis* (Yasuda et al., 2000), *Cycloneda limbifer* Casey, *Ceratomegilla undecimnotata* (Schneider) (Růžička, 2003), *A. bipunctata* (e.g. Fréchette et al., 2004) and *H. convergens* (Michaud & Jyoti, 2007). Some predators such as the syrphid fly *E. balteatus* also react to the presence of conspecific eggs and their chemical residues (Scholz & Poehling, 2000). These responses are presumed to be adaptive because they reduce the risks of cannibalism and competition for offspring (Dixon, 2000)

To summarize, host recognition and acceptance by parasitoids and predators involve contact chemicals perceived after the aphid has been contacted with the antennae, probed with the ovipositor, or tasted with the mouthparts. Compounds present in the aphid cuticle are likely to be more important than components of cornicle secretion for host recognition. Understanding of the chemicals influencing host acceptance is still rudimentary, partly because of the large number of substances potentially involved, and the difficulty of constructing workable bioassays.

INTERACTION AMONG SEMIOCHEMICALS

Above we provided examples where a blend of volatiles was more important for habitat or host location than single compounds, i.e. where additive or synergistic interactions among semiochemicals occurred. However, there is also the possibility of antagonistic interactions among semiochemicals. A well-known example is the interaction between the aphid alarm pheromone EBF and β-caryophyllene, a sesquiterpenoid compound found in many plant species. β-caryophyllene is an EBF-inhibitor,

altering not only the behaviour of the aphids, but also that of predators (Dawson et al., 1984; Mostafavi et al., 1996; Zhu et al., 1999; Al Abassi et al., 2000). The ratio of EBF to β-caryophyllene is important here; a minimum ratio of 1 : 1 elicited a positive response by *C. septempunctata* in a Y-tube olfactometer, while a ratio of 1 : 3 did not significantly attract the predator. The importance of this ratio was also demonstrated for the aphid parasitoid *D. rapae* exposed to the odour of genetically modified *Arabidopsis thaliana* plants which produced increased amounts of EBF relative to β-caryophyllene (Beale et al., 2006).

A new hypothesis for the use of volatiles by insects foraging for their suitable prey was presented by Bruce et al. (2005). In addition to the use of combined compounds in specific ratios, insects appear to time the arrival of these different compounds on their chemical receptors. This could allow natural enemies to differentiate suitable targets from unsuitable ones if the latter emitted the same compounds but with different timing. However, this hypothesis has to be yet demonstrated for aphid natural enemies.

To summarize, both synergistic and antagonistic interactions among semiochemicals have been described and there are likely to be many more, given the fact that the same semiochemicals are involved in many aphid-natural enemy interactions. Testing combinations of semiochemicals for synergistic or antagonistic effect is laborious but would provide a fuller understanding of aphid chemical ecology.

CONCLUSIONS AND FUTURE DIRECTIONS

Plants produce volatile chemicals that are conspicuous to many insects including herbivores and their natural enemies. Chemicals from plants, although less reliable than chemicals from aphids, are important cues that elicit foraging behaviour in many aphid natural enemies, in particular more specialised parasitoids. Currently, there is increasing evidence that volatile blends from aphid-damaged plants play a pivotal role in habitat location by both parasitoids and predators, although only a few model systems have been investigated in detail. Plant changes are probably triggered by elicitors present in the aphid saliva (de Vos et al., 2007). Among the short range cues used by natural enemies are the pheromones of aphids (alarm and sex), and honeydew. For parasitoids, contact kairomones in the aphid cuticle, in particular waxes may be used for host recognition, whereas chemicals within the hemolymph are important for host acceptance. For predators, such compounds may act as a feeding stimulus.

In most cases, one or only a few semiochemicals of importance have been described for a particular system and these often differ among natural enemy species, suggesting that particular plant-aphid-complexes emit specific volatile chemicals. However, there are also examples of redundancy where the same compound is used by many natural enemies, e.g. MHO (Table 1). More studies are needed that submit a particular parasitoid species to different aphid-plant complexes to investigate whether natural enemy responses differ among complexes that produce similar volatile chemicals. Chemical analyses of these volatile blends are needed to investigate subtle differences. Finally, all species are embedded in a network of ecological interactions that shape the evolution of all traits. Understanding the evolution of the semiochemicals requires taking into account the interactions of aphids with plants, competitors, natural enemies and mutualists. For this, more field experiments are needed to unravel the true significance of semiochemicals under natural conditions.

ACKNOWLEDGEMENTS. We gratefully thank two reviewers for extensive helpful comments that have greatly improved previous versions of this manuscript. We thank the Max Planck Society (EH) and the DFG (GK, WWW) for financial support.

REFERENCES

ACAR E.B., MEDINA J.C., LEE M.L. & BOOTH G.M. 2001: Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). *Can. Entomol.* **133**: 389–397.

AL ABASSI S., BIRKETT M.A., PETTERSSON J., PICKETT J.A., WADHAMS L.J. & WOODCOCK C.M. 2000: Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. *J. Chem. Ecol.* **26**: 1765–1771.

BATTAGLIA D., PENNACCHIO F., ROMANO A. & TRANFAGLIA A. 1995: The role of physical cues in the regulation of host recognition and acceptance behavior of Aphidius ervi Haliday (Hymenoptera, Braconidae). *J. Insect Behav.* **8**: 739–750.

BATTAGLIA D., POPPY G., POWELL W., ROMANO A., TRANFAGLIA A. & PENNACCHIO F. 2000: Physical and chemical cues influencing the oviposition behaviour of Aphidius ervi. *Entomol. Exp. Appl.* **94**: 219–227.

BEALE M.H., BIRKETT M.A., BRUCE T.J.A., CHAMBERLAIN K., FIELD L.M., HUTTLY A.K., MARTIN J.L., PARKER R., PHILLIPS A.L., PICKETT J.A., PROSSER I.M., SHEWRY P.R., SMART L.E., WADHAMS L.J., WOODCOCK C.M. & ZHANG Y.H. 2006: Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *PNAS* **103**: 10509–10513.

BEN SAAD A.A. & BISHOP G.W. 1976: Effect of artificial honeydews on insect communities in potato fields. *Envir. Entomol.* **5**: 453–457.

BIRKETT M.A., CAMPBELL C.A.M., CHAMBERLAIN K., GUERRIERI E., HICK A.J., MARTIN J.L., MATTHES M., NAPIER J.A., PETTERSSON J., PICKETT J.A., POPPY G.M., POW E.M., PYE B.J., SMART L.E., WADHAMS G.H., WADHAMS L.J. & WOODCOCK C.M. 2000: New roles for cis-jasmone as an insect semiochemical and in plant defense. *PNAS* **97**: 9329–9334.

BOMBOSCH S. & VOLK S.T. 1966: Selection of the oviposition site by Syrphus corollae F. In Hodek I. (ed.): *The Ecology of Aphidophagous Insects*. Academia Publishing House, Prague, pp. 117–119.

BOO K.S., CHUNG I.B., HAN K.S., PICKETT J.A. & WADHAMS L.J. 1998: Response of the lacewing Chrysopa cognata to pheromones of its aphid prey. *J. Chem. Ecol.* **24**: 631–643.

BOUCHARD Y. & CLOUTIER C. 1985: Role of olfaction in host finding by aphid parasitoid Aphidius nigripes (Hymenoptera, Aphidiidae). *J. Chem. Ecol.* **11**: 801–808.

BRUCE T.J.A., WADHAMS L.J. & WOODCOCK C.M. 2005: Insect host location: a volatile situation. *Trends Plant Sci.* **10**: 269–274.

BUDENBERG W.J. 1990: Honeydew as a contact pheromone for aphid parasitoids. *Entomol. Exp. Appl.* **55**: 139–148.

BUDENBERG W.J. & POWELL W. 1992: The role of honeydew as an ovipositional stimulant for two species of syrphids. *Entomol. Exp. Appl.* **64**: 57–61.

BUDENBERG W.J., POWELL W. & CLARK S.J. 1992: The influence of aphids and honeydew on the leaving rate of searching aphid parasitoids from wheat plants. *Entomol. Exp. Appl.* **63**: 259–264.

CARTER M.C. & DIXON A.F.G. 1984: Honeydew – an arrestant stimulus for Coccinellids. *Ecol. Entomol.* **9**: 383–387.

CHOI M.Y., ROITBERG B.D., SHANI A., RAWORTH D.A. & LEE G.H. 2004: Olfactory response by the aphidophagous gall midge, Aphidoletes aphidimyza to honeydew from green peach aphid, Myzus persicae. *Entomol. Exp. Appl.* **111**: 37–45.

DAWSON G.W., GRIFFITHS D.C., PICKETT J.A., SMITH M.C. & WOODCOCK C.M. 1984: Natural inhibition of the aphid alarm pheromone. *Entomol. Exp. Appl.* **36**: 197–199.

DE VOS M., KIM J.H. & JANDER G. 2007: Biochemistry and molecular biology of Arabidopsis-aphid interactions. *BioEssays* **29**: 871–883.

DICKE M. & SABELIS M.W. 1988: Infochemical terminology: Based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.* **2**: 131–139.

DIXON A.F.G. 1958: The escape responses shown by certain aphids to the presence of the coccinellid Adalia decempunctata (L.). *Trans. R. Entomol. Soc. Lond.* **110**: 319–334.

DIXON A.F.G. 1959: An experimental-study of the searching behavior of the predatory Coccinellid beetle Adalia decempunctata (I). *J. Anim. Ecol.* **28**: 259–281.

DIXON A.F.G. 2000: Foraging behaviour. In Dixon A.F.G. (ed.): *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. Cambridge University Press, Cambridge, pp. 82–129.

DU Y.J., POPPY G.M. & POWELL W. 1996: Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of Aphidius ervi. *J. Chem. Ecol.* **22**: 1591–1605.

DU Y.J., POPPY G.M., POWELL W., PICKETT J.A., WADHAMS L.J. & WOODCOCK C.M. 1998: Identification of semiochemicals released during aphid feeding that attract parasitoid Aphidius ervi. *J. Chem. Ecol.* **24**: 1355–1368.

EVANS E.W. & DIXON A.F.G. 1986: Cues for oviposition by ladybird beetles (Coccinellidae) – Response to aphids. *J. Anim. Ecol.* **55**: 1027–1034.

EVANS E.W. & RICHARDS D.R. 1997: Managing the dispersal of ladybird beetles (Col.: Coccinellidae): Use of artificial honeydew to manipulate spatial distributions. *Entomophaga* **42**: 93–102.

FRANCIS F., LOGNAY G. & HAUBRUGE E. 2004: Olfactory responses to aphid and host plant volatile releases: (*E*)-beta-farnesene an effective kairomone for the predator Adalia bipunctata. *J. Chem. Ecol.* **30**: 741–755.

FRANCIS F., VANDERMOTEN S., VERHEGGEN F., LOGNAY G. & HAUBRUGE E. 2005a: Is the (*E*)-beta-farnesene only volatile terpenoid in aphids? *J. Appl. Entomol.* **129**: 6–11.

FRANCIS F.D., MARTIN T., LOGNAY G. & HAUBRUGE E. 2005b: Role of (*E*)-beta-farnesene in systematic aphid prey location by Episyrphus balteatus larvae (Diptera: Syrphidae). *Eur. J. Entomol.* **102**: 431–436.

FRÉCHETTE B., DIXON A.F.G, ALAUZET C. & HEMPTINNE J.L. 2004: Age and experience influence patch assessment for oviposition by an insect predator. *Ecol. Entomol.* **29**: 578–583.

GABRYS B.J., GADOMSKI H.J., KLUKOWSKI Z., PICKETT J.A., SOBOTA G.T., WADHAMS L.J. & WOODCOCK C.M. 1997: Sex pheromone of cabbage aphid Brevicoryne brassicae: Identification and field trapping of male aphids and parasitoids. *J. Chem. Ecol.* **23**: 1881–1890.

GARDNER S.M. & DIXON A.F.G. 1985: Plant structure and the foraging success of Aphidius rhopalosiphi (Hymenoptera, Aphidiidae). *Ecol. Entomol.* **10**: 171–179.

GLINWOOD R.T., DU Y.J. & POWELL W. 1999: Responses to aphid sex pheromones by the pea aphid parasitoids Aphidius ervi and Aphidius eadyi. *Entomol. Exp. Appl.* **92**: 227–232.

GOFF A.M. & NAULT L.R. 1974: Aphid cornicle secretions ineffective against attack by parasitoid wasps. *Environ. Entomol.* **3**: 565–566.

GONZALES W.L., FUENTES-CONTRERAS E. & NIEMEYER H.M. 1999: Semiochemicals associated to spacing behaviour of the bird cherry-oat aphid Rhopalosiphum padi L. (Hem., Aphididae) do not affect the olfactometric behaviour of the cereal aphid parasitoid Aphidius rhopalosiphi De Stephani-Perez (Hym., Braconidae). *J. Appl. Entomol.* **123**: 413–415.

GRASSWITZ T.R. & PAINE T.D. 1992: Kairomonal effect of an aphid cornicle secretion on Lysiphlebus testaceipes (Cresson) (Hymenoptera, Aphidiidae). *J. Insect Behav.* **5**: 447–457.

GUERRIERI E., PENNACCHIO F. & TREMBLAY E. 1993: Flight behavior of the aphid parasitoid Aphidius ervi (Hymenoptera, Braconidae) in response to plant and host volatiles. *Eur. J. Entomol.* **90**: 415–421.

GUERRIERI E., POPPY G.M., POWELL W., TREMBLAY E. & PENNACCHIO F. 1999: Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of Aphidius ervi. *J. Chem. Ecol.* **25**: 1247–1261.

HAGEN K.S. 1986: Ecosystem analysis: plant cultivar (HPR), entomophagous species and food supplement. In Boethel D.J. & Eikenberry R.D. (eds): *Interactions of Plant Resistance and Parasitoid and Predators of Insects*. Wiley, New York, pp. 151–197.

HAGEN K.S., GREANY P., SAWALL E.F. & TASSAN R.L. 1976: Tryptophan in artificial honeydews as a source of an attractant for adult Chrysopa carnea (Neuroptera, Chrysopidae). *Environ. Entomol.* **5**: 458–468.

HAGEN J.S., SAWALL E.F. JR. & TASSAN R.L. 1971: Use of food sprays to increase effectiveness of entomophagous insects. *Proc. Tall Timbers Conference On Ecological Animal Control By Habitat Management. Feb. 26–28, 1970, Tallahassee, Florida, USA. Vol. 2.* pp. 59–81.

HAGVAR E.B. & HOFSVANG T. 1989: Effect of honeydew and hosts on plant colonization by the aphid parasitoid Ephedrus cerasicola. *Entomophaga* **34**: 495–501.

HAN B.Y. & CHEN Z.M. 2002a: Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, Toxoptera aurantii. *J. Chem. Ecol.* **28**: 2203–2219.

HAN B.Y. & CHEN Z.M. 2002b: Composition of the volatiles from intact and mechanically pierced tea aphid-tea shoot complexes and their attraction to natural enemies of the tea aphid. *J. Agr. Food Chem.* **50**: 2571–2575.

HARDIE J., HICK A.J., HOLLER C., MANN J., MERRITT L., NOTTINGHAM S.F., POWELL W., WADHAMS L.J., WITTHINRICH J. & WRIGHT A.F. 1994: The responses of Praon spp parasitoids to aphid sex-pheromone components in the field. *Entomol. Exp. Appl.* **71**: 95–99.

HARDIE J., NOTTINGHAM S.F., POWELL W. & WADHAMS L.J. 1991: Synthetic aphid sex pheromone lures female parasitoids. *Entomol. Exp. Appl*. **61**: 97–99.

HARMEL N., ALMOHAMAD R., FAUCONNIER M.L., DU JARDIN P., VERHEGGEN F., MARLIER M., HAUBRUGE E. & FRANCIS F. 2007: Role of terpenes from aphid-infested potato on searching and oviposition behavior of Episyrphus balteatus. *Insect Sci.* **14**: 57–63.

HARMON J.P., LOSEY J.E. & IVES A.R. 1998: The role of vision and color in the close proximity foraging behavior of four coccinellid species. *Oecologia* **115**: 287–292.

HATANO E., KUNERT G., BARTRAM S., BOLAND W., GERSHENZON J. & WEISSER W.W. 2008: Do aphid colonies amplify their emission of alarm pheromone? *J. Chem. Ecol.* **34**: 1149–1152.

HEMPTINNE J.L., GAUDIN M., DIXON A.F.G. & LOGNAY G. 2000: Social feeding in ladybird beetles: adaptive significance and mechanism. *Chemoecology* **10**: 149–152.

HOLLER C., MICHA S.G., SCHULZ S., FRANCKE W. & PICKETT J.A. 1994: Enemy-induced dispersal in a parasitic wasp. *Experientia* **50**: 182–185.

IDE T., SUZUKI N. & KATAYAMA N. 2007: The use of honeydew in foraging for aphids by larvae of the ladybird beetle, Coccinella septempunctata L. (Coleoptera: Coccinellidae). *Ecol. Entomol.* **32**: 455–460.

KEMPEMA L.A., CUI X.P., HOLZER F.M., & WALLING L.L. 2007: Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiol.* **143**: 849–865.

KIELTY J.P., ALLENWILLIAMS L.J., UNDERWOOD N. & EASTWOOD E.A. 1996: Behavioral responses of three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with prey and habitat. *J. Insect Behav.* **9**: 237–250.

KUNERT G., OTTO S., ROSE U.S.R., GERSHENZON J. & WEISSER W.W. 2005: Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecol. Lett.* **8**: 596–603.

LAROCCA A., FANTI P., ROMANO V.A., MARSICOVETERE E., ISIDORO N., ROMANI R., RUSCHIONI S., PENNACCHIO F. & BATTAGLIA D. 2007: Functional bases of host-acceptance behaviour in the aphid parasitoid Aphidius ervi. *Physiol. Entomol.* **32**: 305–312.

LE RALEC A. & RABASSE J.M. 1988: Structure, sensory receptors and operation of the ovipositor of three Aphidiidae. In Niemczyk E. & Dixon A.F.G. (eds): *Ecology and Efectiveness of Aphidophaga*. SPB Academic Publishing, The Netherlands, pp. 83–88.

LIBBRECHT R., GWYNN D.M. & FELLOWES M.D.E. 2007: Aphidius ervi preferentially attacks the green morph of the pea aphid, Acyrthosiphon pisum. *J. Insect Behav.* **20**: 25–32.

LIEPERT C. & DETTNER K. 1993: Recognition of aphid parasitoids by honeydew-collecting ants – the role of cuticular lipids in a chemical mimicry system. *J. Chem. Ecol.* **19**: 2143–2153.

LOHMAN D.J., LIAO Q. & PIERCE N.E. 2006: Convergence of chemical mimicry in a guild of aphid predators. *Ecol. Entomol.* **31**: 41–51.

MICHA S.G. & WYSS U. 1996: Aphid alarm pheromone (*E*)-ß-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphidiinae). *Chemoecology* **7**: 132–139.

MICHAUD J.P. & JYOTI J.L. 2007: Repellency of conspecific and heterospecific larval residues to Hippodamia convergens (Coleoptera: Coccinellidae) ovipositing on sorghum plants. *Eur. J. Entomol.* **104**: 399–405.

MICHAUD J.P. & MACKAUER M. 1994: The use of visual cues in host evaluation by Aphidiid wasps. 1. Comparison between 3 Aphidius parasitoids of the pea aphid. *Entomol. Exp. Appl.* **70**: 273–283.

MICHAUD J.P. & MACKAUER M. 1995: The use of visual cues in host evaluation by Aphidiid wasps. 2. Comparison between Ephedrus californicus, Monoctonus paulensis, and Praon pequodorum. *Entomol. Exp. Appl.* **74**: 267–275.

MICHAUD J.P. & QURESHI J.A. 2005: Induction of reproductive diapause in Hippodamia convergens (Coleoptera: Coccinellidae) hinges on prey quality and availability. *Eur. J. Entomol.* **102**: 483–487.

MOSTAFAVI R., HENNING J.A., GARDEA-TORRESDAY J. & RAY I.M. 1996: Variation in aphid alarm pheromone content among glandular and eglandular-haired Medicago accessions. *J. Chem. Ecol.* **22**: 1629–1638.

MURATORI F., LE RALEC A., LOGNAY G. & HANCE T. 2006: Epicuticular factors involved in host recognition for the aphid parasitoid Aphidius rhopalosiphi. *J. Chem. Ecol.* **32**: 579–593.

NAKAMUTA K. 1984: Visual orientation of a ladybeetle, Coccinella septempunctata L. (Coleoptera, Coccinellidae), toward its prey. *Appl. Entomol. Zool.* **19**: 82–86.

NAKAMUTA K. & SAITO T. 1985: Recognition of aphid prey by the lady beetle, Coccinella septempunctata bruckii Mulsant (Coleoptera, Coccinellidae). *Appl. Entomol. Zool.* **20**: 479–483.

NINKOVIC V., AL ABASSI S. & PETTERSSON J. 2001: The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. *Biol. Control* **21**: 191–195.

NORDLUND D.A. & LEWIS W.J. 1976: Termilogy of chemical releasing stimuli in intraspecific and interspecific interactions. *J. Chem. Ecol.* **2**: 211–220.

PAREJA M., MORAES M.C.B., CLARK S.J., BIRKETT M.A. & POWELL W. 2007: Response of the aphid parasitoid Aphidius funebris to volatiles from undamaged and aphid-infested Centaurea nigra. *J. Chem. Ecol.* **33**: 695–710.

PICKETT J.A., WADHAMS L.J., WOODCOCK C.M. & HARDIE J. 1992: The chemical ecology of aphids. *Annu. Rev. Entomol.* **37**: 67–90.

PINTO D.M., BLANDE J.D., NYKANEN R., DONG W.X., NERG A.M. & HOLOPAINEN J.K. 2007: Ozone degrades common herbivore-induced plant volatiles: Does this affect herbivore prey location by predators and parasitoids? *J. Chem. Ecol.* **33**: 683–694.

POWELL W. & ZHANG Z.L. 1983: The reactions of 2 cereal aphid parasitoids, Aphidius uzbekistanicus and Aphidius ervi to host aphids and their food-plants. *Physiol. Entomol.* **8**: 439–443.

POWELL W., PENNACCHIO F., POPPY G.M. & TREMBLAY E. 1998: Strategies involved in the location of hosts by the parasitoid Aphidius ervi Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biol. Control* **11**: 104–112.

READ D.P., FEENY P.P. & ROOT R.B. 1970: Habitat selection by the aphid parasite Diaretiella rapae (Hymenoptera: Braconidae) and hyperparasite Charips brassicae (Hymenoptera: Cynipidae). *Can. Entomol.* **102**: 1567–1578.

RŮŽIČKA Z. 1994: Oviposition-deterring pheromone in Chrysopa oculata (Neuroptera: Chrysopidae). *Eur. J. Entomol.* **91**: 361–370.

RŮŽIČKA Z. 1997: Recognition of oviposition-deterring allomones by aphidophagous predators (Neuroptera: Chrysopidae, Coleoptera: Coccinellidae). *Eur. J. Entomol.* **94**: 431–434.

RŮŽIČKA Z. 2003: Perception of oviposition-deterring larval tracks in aphidophagous coccinellids Cycloneda limbifer and Ceratomegilla undecimnotata (Coleoptera: Coccinellidae). *Eur. J. Entomol.* **100**: 345–350.

SCHOLZ D. & POEHLING H.M. 2000: Oviposition site selection in Episyrphus balteatus. *Entomol. Exp. Appl.* **94**: 149–158.

SCHUSTER D.J. & STARKS K.J. 1974: Response of Lysiphlebus testaceipes in an olfactometer to a host and a non-host insect and to plants. *Environ. Entomol.* **3**: 1034–1035.

SCHWARTZBERG E., KUNERT G., STEPHAN C., DAVID A., ROESE U.S., GERSHENZON J., BOLAND W. & WEISSER W.W. 2008: Real-time analysis of alarm pheromone emission by the pea aphid (Acyrthosiphon pisum). *J. Chem. Ecol.* **34**: 76–81.

SINGH R. & SINHA T.B. 1982: Bionomics of Trioxys (Binodoxys) indicus Subba Rao and Sharma, an aphidiid parasitoid of Aphis craccivora Koch. 13. Host selection by the parasitoid. *J. Appl. Entomol.* **93**: 64–75.

STEIDLE J.L.M. & VAN LOON J.J.A. 2002: Chemoecology of parasitoid and predator oviposition behaviour. In Hilker M. & Meiners T. (eds): *Chemoecology of Insect Eggs and Egg Deposition*. Blackwell, Berlin, pp. 291–318.

VAN EMDEN H.F. 1988: The potential for managing indigenous natural enemies of aphids on field crops. *Philos. Trans. R. Soc. (B)* **318**: 183–201.

VAN EMDEN H.F. & HAGEN K.S. 1976: Olfactory reactions of green lacewing, Chrysopa carnea, to tryptophan and certain breakdown products (Neuroptera-Chrysopidae). *Environ. Entomol.* **5**: 469–473.

VET L.E.M. & DICKE M. 1992: Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37**: 141–172.

VINSON S.B. 1976: Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**: 109–133.

VÖLKL W. 1992: Aphids or their parasitoids: Who actually benefits from ant-attendance? *J. Anim. Ecol.* **61**: 273–281.

WALLING L.L. 2000: The myriad plant responses to herbivores. *J. Plant Growth Regul.* **19**: 195–216.

WEINBRENNER M. & VOLKL W. 2002: Oviposition behaviour of the aphid parasitoid, Aphidius ervi: Are wet aphids recognized as host? *Entomol. Exp. Appl.* **103**: 51–59.

YASUDA H., TAKAGI T. & KOGI G. 2000: Effects of conspecific and heterospecific larval tracks on the oviposition behaviour of the predatory ladybird Harmonia axyridis (Coleoptera: Coccinellidae). *Eur. J. Entomol.* **97**: 551–553.

ZHU J.W., COSSE A.A., OBRYCKI J.J., BOO K.S. & BAKER T.C. 1999: Olfactory reactions of the twelve-spotted lady beetle, Coleomegilla maculata and the green lacewing, Chrysoperla carnea to semiochemicals released from their prey and host plant: Electroantennogram and behavioral responses. *J. Chem. Ecol.* **25**: 1163–1177.

ZHU J., OBRYCKI J.J., OCHIENG S.A., BAKER T.C., PICKETT J.A. & SMILEY D. 2005: Attraction of two lacewing species to volatiles produced by host plants and aphid prey. *Naturwissenschaften* **92**: 277–281.

ZHU J.W. & PARK K.C. 2005: Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator Coccinella septempunctata. *J. Chem. Ecol.* **31**: 1733–1746.

Received September 29, 2007; revised and accepted September 24, 2008

Aphid alarm pheromone mediates avoidance of habitats with increased risk of intraguild predation

Mojtaba Hosseini[1], Eduardo Hatano[2], Ahmad Ashouri[1], Wolfgang W. Weisser[2]

[1]Department of Plant Protection, University of Tehran
[2]Institute of Ecology, Friedrich Schiller University

Submitted to Ecological Entomology on 22 July 2009

ABSTRACT

1. Intraguild predation (IGP) has been described for a number of predator-prey systems, in particular among invertebrates. Because of the substantial mortality inflicted by IGP, there are several examples where the intraguild (IG) prey avoids habitats where the IG predator is present.
2. We investigated the foraging behaviour of the gallmidge *Aphidoletes aphidimyza*, a predator of aphids that is the IG prey in interactions with most other aphid natural enemies. We focused on the role of the aphid alarm pheromone, (*E*)-β-farnesene (EBF), which is given off by aphids attacked by a predator, resulting in dispersal of conspecifics.
3. Using laboratory experiments we show that gallmidge larvae reduce their foraging activities and leave the host plant when exposed to EBF. The response was stronger on aphid infested than on aphid-free plants suggesting that a decrease in foraging success due to aphid movements contributes to the patch-leaving behaviour of gallmidge larvae when aphid alarm pheromone is emitted. EBF application increased head circulation movements of gallmidge larvae in the absence of aphids.
4. In experiments with a high and low density of aphids, female *A. aphidimyza* did not show a reduction in foraging behaviour when exposed to EBF while more eggs were laid at higher aphid density.
5. Because feeding by *A. aphidimyza* larvae itself does not cause unrest in the aphid colony, our results suggest that alarm signalling by aphids induces a behaviour that decreases the risk of IGP to gallmidge larvae.

INTRODUCTION

Intraguild predation (IGP), the killing and consumption of a species that uses similar resources and therefore is a potential competitor (Polis & Holt, 1992), has been well documented for a number of invertebrate and vertebrate predator-predator interactions (Polis *et al.*, 1989; Raymond *et al.*, 2000; Rieger *et al.*, 2004; Rosenheim, 1998; Rosenheim *et al.*, 1995; Sergio *et al.*, 2007; Snyder & Ives, 2001). The aggressor is the intraguild predator (IG predator), the victim is the intraguild prey (IG prey), and the common resource is the extraguild prey (Lucas *et al.*, 1998). Cannibalism among predators is also considered a form of IGP (Hemptinne *et al.*, 2001). Because IGP not only provides an additional food resource for IG predators, but may also reduce inter- or intraspecific competition, IGP is sometimes considered to be an extreme form of competition. Because IG prey populations may suffer substantial mortality due to IGP (Dixon, 2000; Lucas *et al.*, 1998; Sato *et al.*, 2005), there is evidence that in many cases, IG prey tend to avoid habitats where the IG predators are already or potentially present (Nakashima *et al.*, 2004; Nakashima *et al.*, 2006; Sarmento *et al.*, 2007). Such habitat selection has been shown both for IG prey females in their choice of suitable oviposition sites, and for IG prey larvae in their choice of feeding sites. Examples are aphid-feeding ladybirds and lacewings (Agarwala *et al.*, 2003; Ruzicka, 1998, 2001a, b; Sato *et al.*, 2005; Sergio *et al.*, 2007), aphid hymenopteran parasitoids

(Nakashima *et al.*, 2004), dragonflies and damselflies (Ferris & Rudolf, 2007; Mortensen & Richardson, 2008), several species of treefrog (*Hyla*) (Rieger *et al.*, 2004) and various bird species (e.g. Sergio *et al.* 2007).

While in vertebrates such as birds visual detection of IG predators may be common, invertebrate IG prey may often use chemical cues associated with the presence of IG predator for habitat selection (Dicke & Grostal, 2001). For example, there is evidence for oviposition-deterring compounds in the tracks of larvae of ladybird species (Coleoptera: Coccinellidae) that deter females of the same or other ladybird species from laying eggs on a plant (Hemptinne *et al.*, 2001; Ruzicka, 2003, 2006). Hydrocarbons left on the plant by foraging adult ladybirds *Coccinella septempunctata* and *A. bipunctata* also lead to patch-leaving behavior of a number of aphid parasitoid species (*Aphidius, Praon*) (Nakashima *et al.*, 2006). In addition to these non-volatile ladybird tracks volatile cues have been implicated in the IGP avoidance behaviour by the ladybird *Cycloneda sanguinea* but the compounds involved have not yet been identified (Sarmento *et al.*, 2007). In general, we still know little about how IG preys decide to avoid or to leave a patch where the risk of IGP is high. For habitat choice by an IG prey, any chemical cue emitted by an IG predator is potentially a candidate cue to avoid contact with a particular IG predator species. In addition, chemical compounds emitted by the (extraguild) prey, when preyed upon, would also indicate the presence of an aphid predator, but would not be specific to a predator species. The use of such unspecific signals has not been described for IGP systems.

Aphids (Homoptera: Aphididae) are attacked by a large number of predators and parasitoids (Völkl *et al.*, 2007), and hence IGP among aphidophagous guild is frequent (Hindayana *et al.*, 2001; Lucas & Brodeur, 2001; Snyder & Ives, 2001; Snyder & Ives, 2003). One very effective aphid predator that is used frequently in aphid biocontrol is the predatory gallmidge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (Lucas & Brodeur, 1999; Markkula *et al.*, 1979). The rather small and defenseless larvae of *A. aphidimyza* suffer from IGP by many other aphid predators, in particular ladybird larvae and predatory anthocorid bugs (Christensen *et al.*, 2002; Frechette *et al.*, 2008; Hindayana *et al.*, 2001; Lucas & Brodeur, 2001; Lucas *et al.*, 1998). The larva of *A. aphidimyza* is a furtive predator and extracts the aphids' body contents on site without stimulating any significant increased in aphid dropping behaviour that would alert other predators to its presence. In addition, there is evidence that *A. aphidimyza* larvae leave patches where they are preyed upon by other predators (Lucas & Brodeur, 2001; Lucas *et al.*, 1998). In aphids, one important compound that mediates interactions between individuals in the case of predation is the aphid alarm pheromone, (*E*)-β-farnesene (EBF), that is emitted when an aphid is attacked by a predator (Bowers *et al.*, 1972; Kislow & Edwards, 1972; Montgomery & Nault, 1977). EBF triggers various behavioural reactions in aphids: they become more alert, withdraw the stylets from the plant, or drop off their host plants (Montgomery & Nault, 1977; Wohlers, 1980). Because EBF is only emitted after attack, it is an indication for predatory activity in the aphid colony.

In this paper, we use synthetic EBF to investigate the role of aphid alarm pheromone for host selection behaviour of the gallmidge *A. aphidimyza*. The use of EBF to indicate the presence of an IG predator would be interesting as this would be the first example of the use of unspecific (extraguild) prey alarm signaling for the avoidance of IGP. In particular, we test if (1) *A. aphidimyza* larvae change their foraging behaviour in aphid colonies when exposed to EBF, (2)

females of *A. aphidimyza* oviposit in aphid colonies behaviour when exposed to EBF, (3) any effect of EBF on the behaviour of *A. aphidimyza* is mediated by changes in the behaviour of the aphids.

MATERIALS AND METHODS

Experimental conditions

Clones of bean aphid, *Aphis fabae*, were reared and experiments were conducted on 4-week-old broad bean, *Vicia faba*, covered with air-permeable cellophane bags (18.8 × 39 cm). Plants were grown in 10-cm-diameter pots. For the experiments we initiated aphid lines by placing single aphid females on new plants. Descendents of a single foundress were split among treatments in each experiment to account for maternal effects (Kunert & Weisser, 2003) and were always tested on the same day. Rearing of plants, aphids and predators as well as experiments were conducted at 20 ˚C, with a photoperiod of 16:8 L:D and about 75% relative humidity.

Rearing of experimental predators

The predatory midge, *A. aphidimyza* Rondani, was obtained from a commercial supplier (Katz Biotech Services, Germany) as pupae. Flies were hatched by placing the pupae into a dark growth chamber for 48 hours at 20˚C. Some fly couples were kept separately in the test tubes (diameter 100 mm and height 50 mm) to mate for 24 hours and gravid females flies were used in experiments with females. The other adult flies were released onto aphid-infested plants for laying eggs. Nine days after eclosion, the larvae reached the third instar and were used in experiments with larvae.

Experiments with gallmidge larvae

The experiment had two treatments with two factor levels each, in a 2x2 factorial design. One predatory gallmidge larva was released either on an aphid-free or on a plant infested with 10 third or fourth instar *A. fabae* and was exposed to either EBF or a hexane control.

To obtain experimental aphids, two adult aphids from the stem culture were placed on a new broad bean plant to produce 10-12 offspring within 24h. Offspring reached the third or fourth larval stages within six days and were then used in the experiment.

A single larva of *A. aphidimyza* was starved for 5 hours before being placed on a leaf with aphids of the experimental plant inside the cage, using a fine brush. Immediately after placing the larva on the plant, EBF solution (500 ng in 3 µl n-hexane) or only n-hexane were applied using a glass syringe through the cellophane bag to a piece of filter paper held by wire fixed in the soil (Kunert *et al.*, 2005). For the next 15 min (=15 observations), the behaviour of *A. aphidimyza* was observed in one-minute intervals. Larvae displayed one of the following four mutually exclusive behaviours when being on the plant: moving (larval movement), head circulation movements,

resting, and feeding. We also noted when a larva was off the plant at an observation. Aphid behaviour was observed for 1 min after the application of EBF or hexane. We only noted if aphids walked away or dropped from the plant upon application of the solution. Because aphids that dropped first walked for a few they are included in the number of aphids walking.

We calculated the following variables from the predator observation data:
Using the total number of predator observations as a denominator we calculated the *proportion of time spent on plant* and the *proportion of time spent off the plant*. Using the number of observations where the predator was on the plant as a denominator we calculated the *proportion of time spent with head circulation movements* and the *proportion of time spent moving*. For the treatments with aphid-infested plants we calculated the *proportion of time spent feeding*. For aphid-infested plants, we also calculated the *time to first attack* as the number of observations before larva first attacked an aphid.

In total 15 lines (=15 x 4 treatments= 60 replicates) were tested, two to three on a particular day.

Experiment with adult female A. aphidimyza

This experiment had two treatments with two factor levels each, in a 2x2 factorial design. Because preliminary experiments showed that female *A. aphidimyza* only lay eggs on aphid-infested plants, females were released on plants with either 50 (high density) or 5 (low density) aphids. The different densities were chosen to test the effect of EBF on females over a broader range of aphid densities. Female flies and aphids were exposed to either EBF or a hexane control to test their behavioural response and the treatments were repeated twice (eight and 16 h after the start) for test for an influence of alarm pheromone on female reproduction.

To obtain low-density aphid colonies, a single adult of *A. fabae* was introduced on a new bean plant and allowed to produce offspring for 24 hours. Five offspring were left on the plant. To obtain high-density colonies, 10 adult aphids were introduced to a plant for one day and of the produced offspring and about 50 (48-52) were left on the plants. The offspring were used in the experiment when they were six days old. A single mated female of *A. aphidimyza* (17 days old) was released into the cellophane bag using a glass tube (∅15 mm, length 120mm) and immediately EBF solution or n-hexane was applied on a piece of filter paper below the experimental plant. The behaviour of the female was observed every minute for 10 min. We noted the position of the female (flying in the air, on the plant, on the cellophane bag) to calculate the *proportion of time spent on the plant*, the *proportion of time spent on the bag* and the *proportion of time flying* by dividing the number of observations where the female showed a particular behaviour by the total number of observations.

In addition, aphid walking behaviour was recorded for 1 min. Thereafter, females stayed in the cage and were removed 24 hours after the start of the experiment when the total number of eggs laid on the plant was counted. In total there were 27 lines (=27 x 4 treatments= 108 replicates).

Statistical analysis

Results are presented as means ± standard error in all cases. For the comparisons of proportional data (gallmidge behavioural responses), gallmidge reproduction and numbers of aphid movements in the larva and female experiments, generalized linear models (GLM) were performed by including the day of experiment (block) as a random effect factor (Crawley, 2002; Littell *et al.*, 1996). In all cases differences in treatments were assessed by comparing Bonferroni's test of means. In addition, to determine how aphid movements affected the proportion of time larvae spent off the plant, linear regression analysis was used. The analyses were performed using SAS, version 8.0. (SAS, 2000).

RESULTS

Experiments with gallmidge larvae

Gallmidge larvae started to search for food in all replicates. Resting was very rare and was not further analysed. The proportion of time that *A. aphidimyza* larvae were moving on the plant was not significantly influenced by aphid presence (Table 1). When larvae were exposed to EBF, the proportion of time spent moving increased compared to the hexane treatment both with and without aphids on the plant (Fig. 1, Table 1). For larval head circulation movements there was a significant interaction between alarm pheromone treatment and aphid presence (Fig. 1, Table 1): when aphids were present, there was no difference in head circulation movements between the EBF treatment and the hexane control, but in the absence of aphids, larvae showed more head circulation movements in the EBF treatment. The highest proportion of head circulation movements was observed in replicates with EBF application and without aphids, and the lowest in replicates without aphids and hexane application (Fig. 1).

When aphids were on the plant, *A. aphidimyza* larvae spent a higher proportion of time feeding on aphids when exposed to hexane (0.25 ± 0.07) compared to the EBF treatment (0.07 ± 0.02, $F_{1,14}$ = 10.69, P < 0.01). There was no significant difference in the time to first attack between the EBF (11 ± 0.51 min) and hexane treatment (7.79 ± 1.89 min, $F_{1,3}$ = 3.42, P = 0.16), although only 9 lines where larvae were feeding could be evaluated.

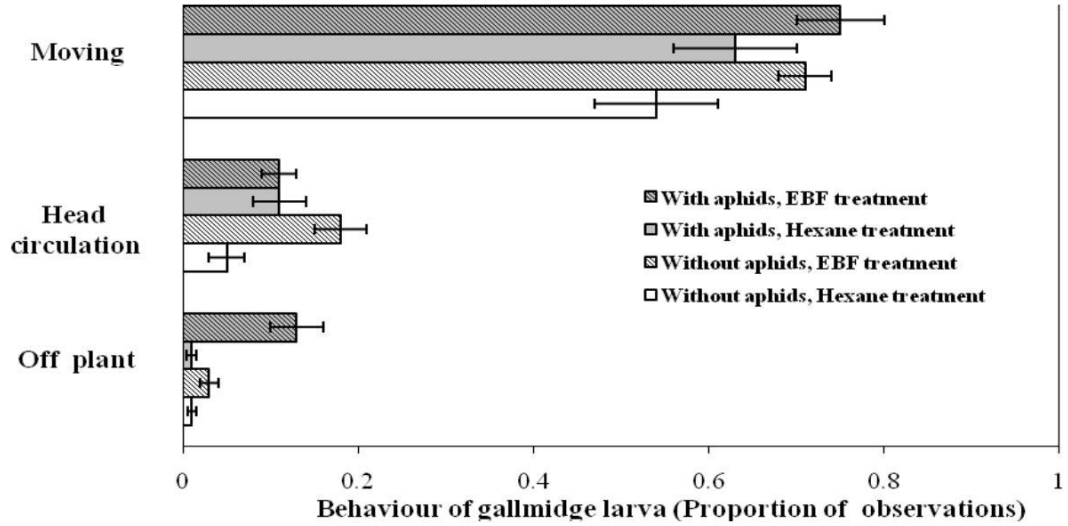

Figure 1. Effects of aphid alarm pheromone and aphid presence on gallmidge behaviour in the experiment with gallmidge larvae. *N* = 15 for each treatment.

Table 1. Statistical analyses of behaviour of *A. aphidimyza* larva in experiment 1. The predator larva was offered either aphid-free plant or aphid-infested plant and was exposed to EBF or hexane. Time (block) was included in the model as a random effect factor. For definitions of behavioural states, see materials and methods.

Behaviour state	Source of variation	SS	MS	F	P
Proportion of time moving	Time of experiment (Block)	1.17	0.08	2.16	0.02
(R^2 = 0.51)	Aphid presence	0.008	0.008	0.21	0.64
	Alarm pheromone	0.28	0.28	7.45	0.009
	Aphid presence * alarm pheromone	0.05	0.05	1.49	0.22
Proportion of time head Circulation movements	Time (Block)	0.16	0.01	1.59	0.13
(R^2 = 0.46)	Aphid presence	0.001	0.001	0.17	0.67
	Alarm pheromone	0.05	0.05	6.68	0.01
	Aphid presence * alarm pheromone	0.07	0.07	8.15	0.006
Proportion of time off plant	Time (Block)	0.11	0.008	1.15	0.34
(R^2 = 0.47)	Aphid presence	0.03	0.03	4.74	0.03
	Alarm pheromone	0.07	0.07	9.97	0.002
	Aphid presence * alarm pheromone	0.04	0.04	6.47	0.01

In the EBF treatment with aphids gallmidge larvae left the plant in 7 out of the 15 replicates, whereas in the other treatment combinations this behaviour was only observed in between one (hexane with aphids) and four (EBF without aphids) replicates. No larvae returned to the plant after leaving it. There was a significant interaction between pheromone treatment and aphid presence for the proportion of time gallmidge larvae were off the plant: the proportion was highest in the EBF treatments on plants with aphids (Fig. 1, Table 1).

On average, 2.8 ± 0.3 aphids walked upon application of EBF compared to 0.5 ± 0.1 after hexane application ($F_{1,14}$ = 85.75, P < 0.001). A linear regression showed a positive relationship between aphid walking behaviour and the proportion of time that gallmidge larvae spent off the plant (treatments with aphids only, R^2 = 0.30 , P = 0.04, Fig. 2).

For some variables, there were differences among blocks in the response to the treatments (Table 1).

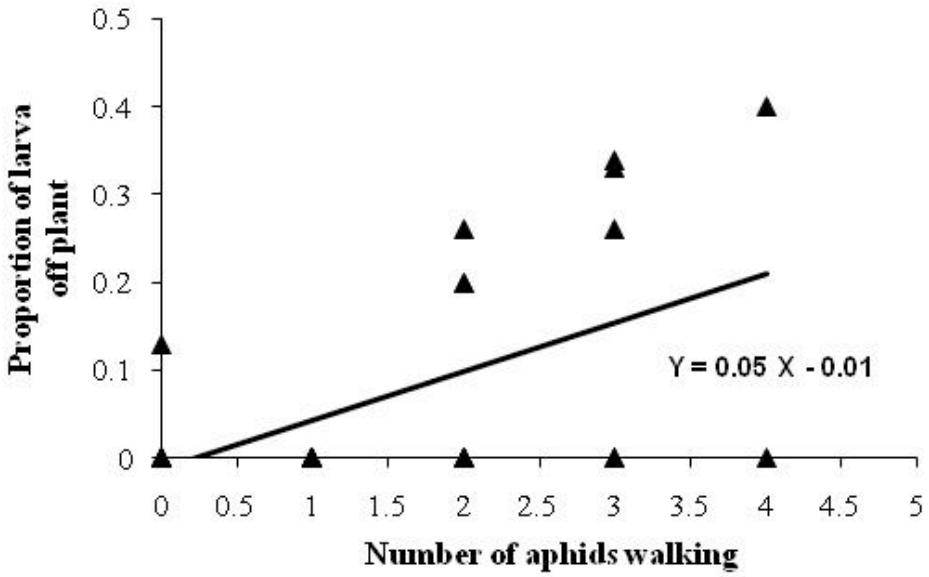

Figure 2: Relationship between the number of aphids responding after EBF application and larval gallmidge plant-leaving behaviour (R^2 = 0.30, P = 0.04, N = 30).

Experiments with gallmidge adults

Female behaviour was strongly affected by aphid density. At higher aphid density, females spent more time on the plant at low aphid density and the reverse was true for the time spent on the bag surrounding the plant (Fig. 3, Table 2). In contrast, the pheromone treatment had no effect on female behaviour and the interaction between pheromone treatment and aphid density was also not significant (Table 2).

Aphids responded to the EBF treatment by initiating walking behaviour and there was a significant pheromone * aphid density interaction (Fig. 4a, $F_{1,78}$ = 52.66, P < 0.01). In the EBF treatment, more aphids walked in the higher aphid density treatment whereas there were little movements were observed in the hexane treatment independent of aphid density.

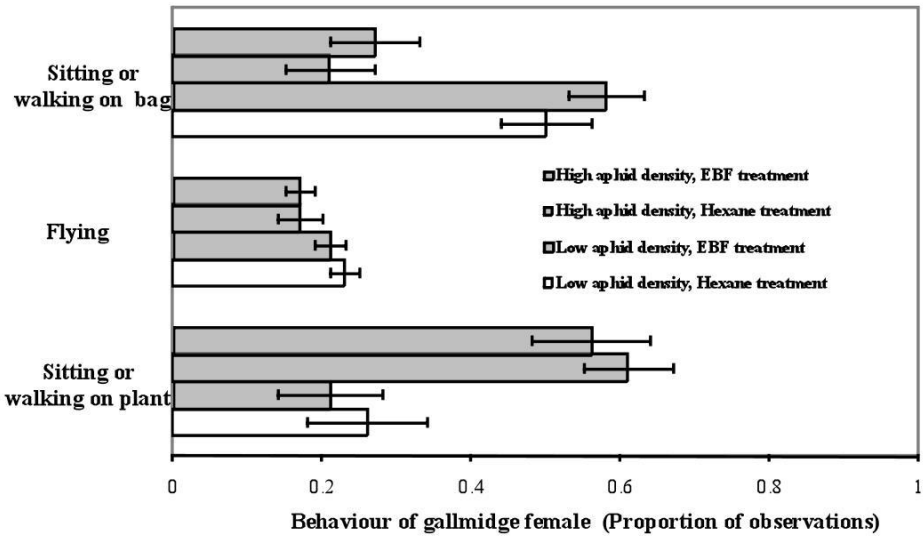

Figure 3: Effects of aphid alarm pheromone and aphid density on adult female behaviour in the *A. aphidimyza* female experiment. *N* = 27 for each treatment.

Table 2. Statistical analyses in experiment 2 with female *A. aphidimyza*. Females were exposed to low/high density of aphids on the plant, and to EBF or a hexane control in a fully factorial design. N=27 for each treatment. Time (block) was included in the model as a random effect factor.

Behavioural state	Source of variation	SS	MS	F	P
Proportion of time on plant (R^2=0.42)	Time of experiment (Block)	4.27	0.16	1.30	0.18
	Aphid density	3.27	3.27	25.81	0.001
	Alarm pheromone	0.07	0.07	0.57	0.45
	Aphid density * alarm pheromone	0.01	0.01	0.03	0.90
Proportion of time flying (R^2=0.40)	Time (Block)	0.95	0.04	1.89	0.01
	Aphid density	0.06	0.06	3.47	0.06
	Alarm pheromone	0.008	0.008	0.38	0.53
	Aphid density * alarm pheromone	0.002	0.002	0.12	0.73
Proportion of time resting on bag (R^2=0.45)	Time (Block)	2.8	0.11	1.34	0.16
	Aphid density	2.4	2.4	29.8	0.001
	Alarm pheromone	0.12	0.12	1.57	0.21
	Aphid density * alarm pheromone	0.005	0.005	0.06	0.81

Fifty seven out of the 108 females laid eggs in the experiment. Females laid significantly more eggs in the high aphid density treatments (Fig. 4b, $F_{1,78} = 10.59$, $P < 0.01$). In contrast, the pheromone treatment did not affect the number of eggs laid ($F_{1,78} = 1.86$, $P = 0.18$) and the interaction between aphid density and pheromone treatment was also not significant ($F_{1,78} = 0.01$, $P = 0.93$). For some variables there were differences among blocks in the response to the treatment (Table 2, for female oviposition and aphid movements data not shown).

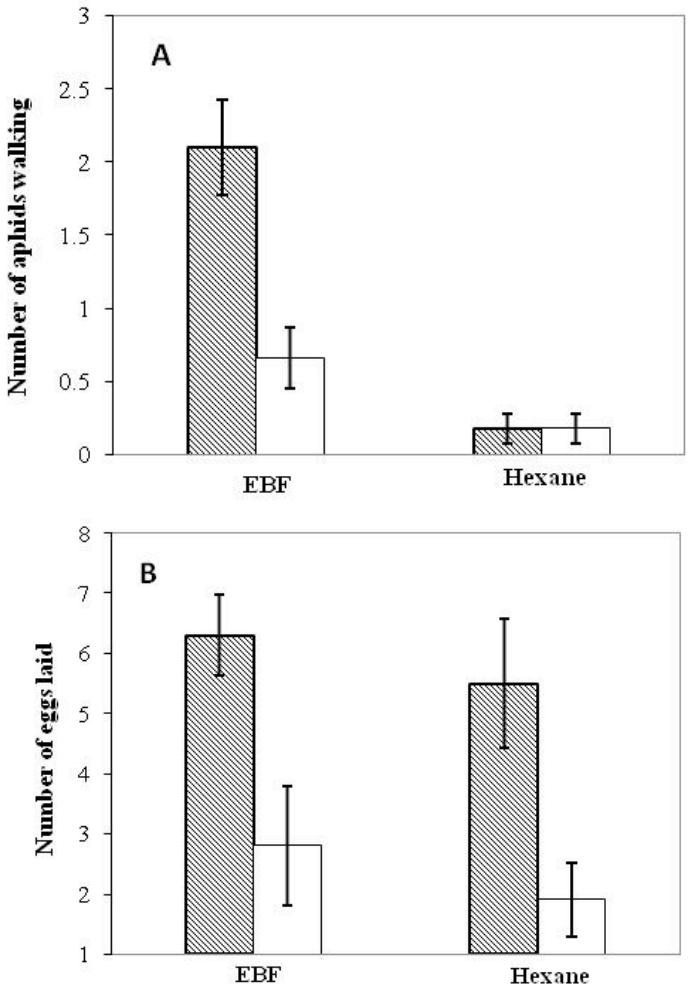

Figure 4: Behaviour of aphids and adult gallmidges in the experiment with female adult *A. aphidimyza*. (A) Number of aphids responding after application of EBF or hexane. (B) Oviposition behaviour of females. *N* = 27 for each treatment.

DISCUSSION

The main result of our study is that larvae, but not adults, of the predatory gallmidge *A. aphidimyza* react to the presence of aphid alarm pheromone by changing their foraging behaviour. Larvae increase their movements resulting in an increased probability in leaving the host plant. Importantly, this effect was observed both when aphids were feeding on the plants and when they were not, suggesting that larvae do perceive EBF. This was also indicated by the head circulation

movements, an orientative behaviour of gallmidge larvae; it strongly increased on plants without aphids after EBF application. In addition, there was a reenforcing affect of aphid behaviour in the treatments where both gallmidge larvae and aphids were present on the plant: the walking responses of aphids decreased larval foraging success that contributed to the plant-leaving response of gallmidge larvae to EBF application. This is supported by the positive relationship between the number of aphids moving and gallmidge time off the plant (Fig. 2). Predatory gallmidges are stealthy predators and larvae approach their victim by inconspicuous creeping movements and subdue them by injecting a paralyzing toxin, thereby deactivating behavioural defences of the prey. Gallmidge feeding itself does not stimulate any significant increase in dropping behaviour or movements of the remaining aphids in the colony (Klingauf, 1967; Lucas & Brodeur, 2001). Thus, any increase in aphid escape behaviour is evidence for the action of a different aphid predator on the plant. Because most of these predators act as IG predator in the interaction with gallmidges e.g. (Christensen *et al.*, 2002; Hindayana *et al.*, 2001; Lucas *et al.*, 1998), the increase in aphid movements therefore indicates a risk of intraguild predation for the gallmidge larvae. By leaving plants where aphids start to move around gallmidge larvae not only leave an unprofitable patch but they also decrease the risk of becoming a victim of IGP.

In contrast, female *A. aphidimyza* searching behaviour and the number of eggs laid within 24 hours were not affected by the application of aphid alarm pheromone. Instead, females responded to an increase in aphid density on the host plant by increasing residence time and oviposition rate. Thus, females respond positively to the likelihood of increasing their reproductive success (Choi *et al.*, 2004; El-Titi, 1973; Lucas & Brodeur, 1999; Stewart & Walde, 1997), but they do not react towards possible risks of their offspring. A possible explanation for the lack of response, apart from a possible inability to perceive EBF, is that EBF emission is not a reliable indicator for the future risk of IGP for the gallmidge offspring. *A. aphidimyza* females do also not respond to the presence of adult or larvae of the coccinellid predator *Coleomegilla maculata*, a particular IG predator (Lucas & Brodeur, 1999). On the other hand, female gallmidges are able to recognize the presence of conspecific gallmidge larvae. When aphid colonies are exposed to *A. aphidimyza* larvae or to water extracts of larvae, female gallmidges lay significantly fewer eggs in such colonies (Ruzicka & Havelka, 1998). These conflicting results need further attention. It is possible that the time-delay between egg-laying and the hatching of the larvae makes an avoidance of currently predator-occupied patches non-adaptive, as many aphid predators stay only for a short time in aphid colonies (Minoretti & Weisser, 2000). With respect to their ability of perceiving EBF, a number of studies have suggested that female midges use honeydew as a cue in the process of prey location and do not use plant volatiles or odours from the aphids themselves (Choi *et al.*, 2004; El-Titi, 1973; El-Titi, 1974; Havelka & Syrovatka, 1991; Mansour, 1976; Wilbert, 1974).

Intraguild predation is widespread in aphidophagous guilds and represents an important mortality factor for aphid predators (Arim & Marquet, 2004; Lucas, 2005; Müller *et al.*, 1999; Rosenheim *et al.*, 1993). We have shown that emission of the aphid alarm pheromone EBF not only alerts aphids but also results in a change in the behaviour of predatory gallmidge larvae. Because in our experiment with aphids and gallmidge larvae we could not fully disentangle the effects of EBF perception and aphid movements on larvael behaviour, one could argue that

gallmidge larvae mainly or solely responded to a decrease in their feeding success and not to the potential presence of another predator on the plant. In this case the patch-leaving behaviour would be an exaptation (Gould & Vrba, 1982) with respect to its role in reducing the risk of IGP for gallmidge larvae, i.e. a behaviour that evolved for a particular reason but also serves another purpose. We suggest, however, that the fast leaving of the plant without a thorough search for hosts argues for a role of the movement behaviour in the escape from IG predator.

Our study provides evidence for aphid alarm pheromone mediating intraguild predator avoidance, which, to our knowledge, provides the first example for a role of an unspecific (extraguild) prey alarm signal in the avoidance of IGP by the intraguild prey. Interestingly, in the interaction between aphids and gallmidges EBF may be classified as a synomone (Vet & Dicke, 1992) as it provides benefits to both the producer and the receiver of the signal: for gallmidge larvae the risk of IGP is reduced while the leaving of gallmidges also provides benefits for the aphids because their predation pressure is reduced.

ACKNOWLEDGEMENTS

We thank Ingrid Jakobi for help in the rearing of plants and animals. Support from the Deutsche Forschungsgemeinschaft (DFG) and the Ministry of Science, Research and Technology of Iran (for MH) is acknowledged. We thank Katz Biotech Services for the provision of predatory gallmidges.

REFERENCES

Agarwala, B.K., Bardhanroy, P., Yasuda, H., & Takizawa, T. (2003) Effects of conspecific and heterospecific competitors on feeding and oviposition of a predatory ladybird: a laboratory study. *Entomologia Experimentalis et Applicata*, **106**, 219-226.

Arim, M. & Marquet, P.A. (2004) Intraguild predation: a widespread interaction related to species biology. *Ecology Letters*, **7**, 557–564.

Bowers, W.S., Webb, R.E., Nault, L.R., & Dutky, S.R. (1972) Aphid alarm pheromone: Isolation, identification, synthesis. *Science*, **177**, 1121-1122.

Choi, M.Y., Roitberg, B.D., Shani, A., Raworth, D.A., & Lee, G.H. (2004) Olfactory response by the aphidophagous gall midge, *Aphidoletes aphidimyza* to honeydew from green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **111**, 37–45.

Christensen, R.K., Enkegaard, A., & Brodsgaard, H.F. (2002) Intraspecific interactions among the predators *Orius majusculus* and *Aphidoletes aphidimyza*. *IOBC/WPRS Bulletin*, **25**, 57-60.

Crawley, M.J. (2002) *Statistical computing*. John Wiley & Sons Ltd, Chichester, West Sussex.

Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods: An ecological perspective. *Annu. Rev. Ecol. Syst.*, **32**, 1-23.

Dixon, A.F.G. (2000). Foraging behaviour. In *Insect predator-prey dynamics: Ladybird beetles and biological control* (ed. by A.F.G. Dixon), pp. 82-129. Cambridge University Press, Cambridge.

El-Titi, A. (1973) Einflüsse von Beutedichte und Morphologie der Wirtsplanze auf die Eiablage von *Aphidoletes aphidimyza* (Rond), (Diptera: Itonididae). *Zeitschrift für Angewandte Entomologie*, **72**, 400–415.

El-Titi, A. (1974) Zur Auslösung der Eiablage bei der aphidophagen Gallmücke *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae). *Entomologia Experimentalis et Applicata*, **17**, 9-21.

Ferris, G. & Rudolf, V.W. (2007) Response of larval dragonflies to conspecific and heterospecific predator cues. *Ecological Entomology*, **32**, 283-288.

Frechette, B., Larouche, F., & Lucas, E. (2008) *Leucopis annulipes* larvae (Diptera: Chamaemyiidae) use a furtive predation strategy within aphid colonies. *European Journal of Entomology*, **105**, 399-403.

Gould, S.J. & Vrba, E.S. (1982) Exaptation - a missing term in the science of form. *Paleobiology* **8**, 4-15.

Havelka, J. & Syrovatka, O. (1991) Stimulatory effect of the honeydew of several species on the females of gall midge *Aphidoletes aphidimyza* (Rodani) (Diptera, Cecidomyiidae) – Electroantennographic studies. *Journal of Applied Entomology*, **112**, 341–344.

Hemptinne, J.L., Lognay, G., Doumbia, M., & Dixon, A.F.G. (2001) Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Chemoecology*, **11**, 43-47.

Hindayana, D., Meyhofer, R., Scholz, D., & Poehling, H.M. (2001) Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera : Syrphidae) and other aphidophagous predators. *Biological Control*, **20**, 236-246.

Kislow, C. & Edwards, L.J. (1972) Repellent odour in aphids. *Nature*, **235**, 108-109.

Klingauf, F. (1967) Abwehr- und Meidereaktionen von Blattla üsen (Aphididae) bein Bedrohung durch Raüber und parasiten. *Zeitschrift für Angewandte Entomologie*, **60**, 269–317

Kunert, G., Otto, S., Rose, U.S.R., Gershenzon, J., & Weisser, W.W. (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters*, **8**, 596-603.

Kunert, G. & Weisser, W.W. (2003) The interplay between density- and trait-mediated effects in predator-prey interactions: A case study in aphid wing polymorphism. *Oecologia*, **135**, 304-312.

Littell, R.C., Milliken, G.A., Stroup, W.W., & Wolfinger, R.D. (1996) *SAS System for Mixed Models*. SAS Institute Inc., Cary, North Carolina.

Lucas, E. (2005) Intraguild predation among aphidophagous predators. *European Journal of Entomology*, **102**, 351-364.

Lucas, E. & Brodeur, J. (1999) Oviposition site selection by the predatory midge *Aphidoletes aphidimyza* (Diptera : Cecidomyiidae). *Environmental Entomology*, **28**, 622-627.

Lucas, E. & Brodeur, J. (2001) A fox in sheep's clothing: Furtive predators benefit from the communal defense of their prey. *Ecology*, **82**, 3246-3250.

Lucas, E., Coderre, D., & Brodeur, J. (1998) Intraguild predation among aphid predators: Characterization and influence of extraguild prey density. *Ecology*, **79**, 1084-1092.

Mansour, M.H. (1976) Some factors influencing egg laying and site of oviposition by *Aphidoletes aphidimyza* (Dipt. Cecidomyiidae). *Entomophaga*, **21**, 281–288.

Markkula, M., Tiittanen, K., Hämäläinen, M., & Forsberg, A. (1979) The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. *Annales Entomologica Fennica*, **45**, 89- 98.

Minoretti, N. & Weisser, W.W. (2000) The impact of individual ladybirds (*Coccinella septempunctata*, Coleoptera: Coccinellidae) on aphid colonies. *European Journal of Entomology*, **97**, 475-479.

Montgomery, M.E. & Nault, L.R. (1977) Comparative response of aphids to the alarm pheromone, (E)-beta-farnesene. *Entomologia Experimentalis et Applicata*, **22**, 236-242.

Mortensen, L. & Richardson, J.M.L. (2008) Effects of chemical cues on foraging in damselfly larvae, *Enallagma antennatum*. *Journal of Insect Behavior*, **21**, 285-295.

Müller, C.B., Adriaanse, I.C.T., Belshaw, R., & Godfray, H.C.J. (1999) The structure of an aphid-parasitoid community. *Journal of Animal Ecology*, **68**, 346–370.

Nakashima, Y., Birkett, M.A., Pye, B.J., Pickett, J.A., & Powell, W. (2004) The role of semiochemicals in the avoidance of the seven-spot ladybird, *Coccinella septempunctata*, by the aphid parasitoid, *Aphidius ervi*. *Journal of Chemical Ecology*, **30**, 1103-1116.

Nakashima, Y., Birkett, M.A., Pye, B.J., & Powell, W. (2006) Chemically mediated intraguild predator avoidance by aphid parasitoids: Interspecific variability in sensitivity to semiochemical trails of ladybird predators. *Journal of Chemical Ecology*, **32**, 1989-1998.

Polis, G.A. & Holt, R.D. (1992) Intraguild Predation - the Dynamics of Complex Trophic Interactions. *Trends in Ecology and Evolution*, **7**, 151-154.

Polis, G.A., Myers, C.A., & Holt, R.D. (1989) The Ecology and Evolution of Intraguild Predation - Potential Competitors That Eat Each Other. *Annual Review of Ecology and Systematics*, **20**, 297-330.

Raymond, B., Darby, A.C., & Douglas, A.E. (2000) Intraguild predators and the spatial distribution of a parasitoid. *Oecologia*, **124**, 367-372.

Rieger, J.F., Binckley, C.A., & Resetarits, W.J. (2004) Larval performance and oviposition site preference along a predation gradient. *Ecology*, **85**, 2094-2099.

Rosenheim, J.A. (1998) Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43**, 421-447.

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., & Jaffee, B.A. (1995) Intraguild Predation among Biological-Control Agents - Theory and Evidence. *Biological Control*, **5**, 303-335.

Rosenheim, J.A., Wilhoit, L.R., & Armer, C.A. (1993) Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia*, **96**, 439-449.

Ruzicka, Z. (1998) Further evidence of oviposition-deterring allomone in chrysopids (Neuroptera : Chrysopidae). *European Journal of Entomology*, **95**, 35-39.

Ruzicka, Z. (2001a) Oviposition responses of aphidophagous coccinellids to tracks of ladybird (Coleoptera: Coccinellidae) and lacewing (Neuroptera: Chrysopidae) larvae. *European Journal of Entomology*, **98**, 183-188.

Ruzicka, Z. (2001b) Response of chrysopids (Neuroptera) to larval tracks of aphidophagous coccinellids (Coleoptera). *European Journal of Entomology*, **98**, 283-285.

Ruzicka, Z. (2003) Perception of oviposition-deterring larval tracks in aphidophagous coccinellids *Cycloneda limbifer* and *Ceratomegilla undecimnotata* (Coleoptera : Coccinellidae). *European Journal of Entomology*, **100**, 345-350.

Ruzicka, Z. (2006) Oviposition-deterring effects of conspecific and heterospecific larval tracks on *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). *European Journal of Entomology*, **103**, 757-763.

Ruzicka, Z. & Havelka, J. (1998) Effects of oviposition-deterring pheromone and allomones on *Aphidoletes aphidimyza* (Diptera : Cecidomyiidae). *European Journal of Entomology*, **95**, 211-216.

Sarmento, R.A., Venzon, M., Pallini, A., Oliveira, E.E., & Janssen, A. (2007) Use of odours by *Cycloneda sanguinea* to assess patch quality. *Entomologia Experimentalis et Applicata*, **124**, 313-318.

SAS, I. (2000) *SAS/Stat User's Guide, Version 8.0.* SAS Institute.

Sato, S., Yasuda, H., & Evans, E.W. (2005) Dropping behaviour of larvae of aphidophagous ladybirds and its effects on incidence of intraguild predation: interactions between the intraguild prey, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.), and the intraguild predator, *Harmonia axyridis* Pallas. *Ecological Entomology*, **30**, 220-224.

Sergio, F., Marchesi, L., Pedrini, P., & Penteriani, V. (2007) Coexistence of a generalist owl with its intraguild predator: distance-sensitive or habitat-mediated avoidance? *Animal Behavior*, **74**, 1607-1616.

Snyder, W.E. & Ives, A.R. (2001) Generalist predators disrupt biological control by a specialist parasitoid. *Ecology*, **82**, 705-7

Snyder, W.E. & Ives, A.R. (2003) Interactions between specialist and generalist natural enemies: Parasitoids, predators, and pea aphid biocontrol. *Ecology*, **84**, 91-107.

Stewart, H.C. & Walde, S.J. (1997) The dynamics of *Aphis pomi* de Geer (Homoptera: Aphididae) and its predator, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), on apple in Nova Scotia. *The Canadian Entomologist*, **129**, 627–636.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141-172.

Völkl, W., Mackauer, M., Pell, J.K., & Brodeur, J. (2007). Predators, parasitoids and pathogens. In *Aphids as crop pests* (ed. by H.F. VanEmden & R. Harrisson), pp. 187-234. CAB International, Wallingford.

Wilbert, H. (1974) The perception of its prey by newly hatched larvae of *Aphidoletes aphidimyza* (Cecidomyiidae). *Entomophaga*, **19**, 173–181.

Wohlers, P. (1980) Die Fluchtaktion der Erbenlaus *Acyrthosiphon pisum* ausgelöst durch Alarmpheromon und zusätzliche Reize. *Entomologia Experimentalis et Applicata*, **27**, 156-168.

Do aphid colonies amplify their emission of alarm pheromone?

Eduardo Hatano[1], Grit Kunert[2], Stefan Bartram[3], Wilhelm Boland[3], Jonathan Gershenzon[2], Wolfgang W. Weisser[1]

[1]Institute of Ecology, Friedrich Schiller University
[2]Biochemistry Department, Max Planck Institute for Chemical Ecology
[3] Bioorganic Chemistry Department, Max Planck Institute for Chemical Ecology

Published in the *Journal of Chemical Ecology* 34, 1149-1152 (2008).

J Chem Ecol (2008) 34:1149–1152
DOI 10.1007/s10886-008-9527-y

RAPID COMMUNICATION

Do Aphid Colonies Amplify their Emission of Alarm Pheromone?

Eduardo Hatano · Grit Kunert · Stefan Bartram · Wilhelm Boland · Jonathan Gershenzon · Wolfgang W. Weisser

Received: 8 April 2008 / Accepted: 21 July 2008 / Published online: 14 August 2008

Abstract When aphids are attacked by natural enemies, they emit alarm pheromone to alert conspecifics. For most aphids tested, (*E*)-β-farnesene (EBF) is the main, or only, constituent of the alarm pheromone. In response to alarm pheromone, alerted aphids drop off the plant, walk away, or attempt to elude predators. However, under natural conditions, EBF concentration might be low due to the low amounts emitted, to rapid air movement, or to oxidative degradation. To ensure that conspecifics are warned, aphids might conceivably amplify the alarm signal by emitting EBF in response to EBF emitted by other aphids. To examine whether such amplification occurs, we synthesized deuterated EBF (DEBF), which allowed us to differentiate between applied and aphid-derived chemical. Colonies of *Acyrthosiphon pisum* were treated with DEBF, and headspace volatiles were collected and analyzed for evidence of aphid-derived EBF. No aphid-derived EBF was detected, suggesting that amplification of the alarm signal does not occur. We discuss the disadvantages of alarm signal reinforcement.

This paper and the preceding paper by Verheggen et al. on the same topic were received and processed essentially simultaneously.

E. Hatano · W. W. Weisser (✉)
Institute of Ecology, Friedrich-Schiller University,
Dornburger str. 159,
07743 Jena, Germany
e-mail: wolfgang.weisser@uni-jena.de

G. Kunert · S. Bartram · W. Boland · J. Gershenzon
Max Planck Institute for Chemical Ecology,
Hans-Knöll str. 8,
07745 Jena, Germany

Keywords Aphid Alarm Pheromone · (*E*)-β-farnesene · Pea Aphid · *Acyrthosiphon pisum* · Synthesis · Deuterium labeling

Introduction

Aphids (Homoptera: Aphididae) are attacked by many different predatory insects and have evolved an efficient defense behavior. When disturbed, they release cornicle droplets that contain a mixture of triglycerides and alarm pheromones (Nault et al. 1973).

The alarm pheromones of many aphid species are, in most cases, single terpenes or mixtures of terpenes (Pickett and Glinwood 2007). For some species, such as the pea aphid, *Acyrthosiphon pisum*, the sesquiterpene (*E*)-β-farnesene (EBF) is the only compound in the alarm pheromone (Francis et al. 2005). The amounts of EBF emitted by individual pea aphids are small, and this may be exacerbated by chemical oxidation or the concentration of the pheromone being diluted rapidly by air movement. Few studies have attempted to investigate emission dynamics of aphid alarm pheromone (Schwartzberg et al. 2008). In particular, it is unclear whether the emission is confined to the attacked aphid or whether the signal is reinforced by members of the colony. The emission of alarm pheromone by aphids that perceive the chemical, but are not attacked, would amplify the signal and presumably warn more individuals in a colony. However, such amplified emission would also expose inconspicuous aphids to natural enemies or unnecessarily alert aphids that are not at risk.

In this paper, we report on a series of experiments in which we use a deuterated (*E*)-β-farnesene derivative

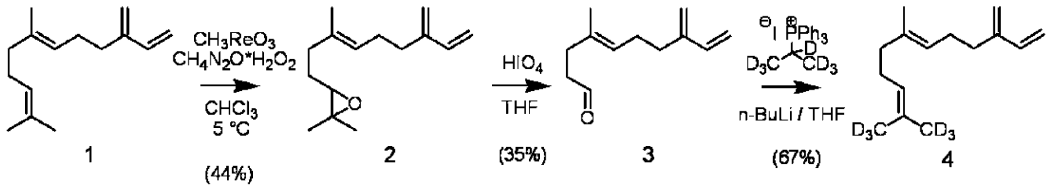

Fig. 1 Synthesis of [12,12,12,13,13,13]-$^2H_6$-(*E*)-β-farnesene

(DEBF), labeled on the geminal dimethyl group, to investigate signal reinforcement in the pea aphid.

Methods and Materials

Synthesis of Deuterated EBF Following Cravotto et al. (2004) (Fig. 1), epoxidation of (*E*)-β-farnesene (**1**) (408.70 mg, 2 mmol), employing methyltrioxorhenium (VII) and urea-hydrogen peroxide adduct in $CHCl_3$ at 5°C, gave a mixture of mono- and di-oxiranes. The 10,11-epoxy-(*E*)-β-farnesene (**2**) (180.3 mg, 44%, R_f 0.48) was separated on Florisil 100–200 (petroleum ether 40–60/ether 40:1 *v/v*). As specified by Fielder and Rowan (1994) (Fig. 1), the epoxide **2** was cleaved with periodic acid to give (*E*)-4-methyl-8-methylenedeca-4,9-dienal (**3**) (49.1 mg, 35%, R_f 0.27), which was purified on silica 60 (petroleum ether 40–60/ether 40:1 *v/v*). Wittig reaction of **3** with d_7-isopropyltriphenylphosphonium iodide produced the d_6-(*E*)-β-farnesene (**4**) ([12,12,12,13,13,13]-$^2H_6$-(*E*)-β-farnesene; DEBF) (221.60 μg, 67%, R_f 0.60). Purification of **4** was carried out by flash chromatography on silica gel (silica 60, Merck, Darmstadt, Germany) with pentane. The chemical purity of DEBF after chromatographic purification was 97% as determined by gas chromatography-mass spectrometry (GC-MS).

NMR Data of Deuterated EBF ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm)=1.53 (s, 3H), 1.89–1.94 (m, 2H), 1.97–2.03 (m, 2H), 2.08–2.19 (m, 4H), 4.92 (d, *J*=1.3 Hz, 1H), 4.94 (d, *J*=1.3 Hz, 1H), 4.98 (d, *J*=10.8 Hz, 1H), 5.03 (t, *J*=6.8 Hz, 1H), 5.09 (tq, *J*=6.8, 1.0 Hz, 1H), 5.17 (d, *J*=17.7 Hz, 1H), 6.31 (dd, *J*=17.7, 10.8 Hz, 1H).

Aphid Lines We used a red clone (BP) of the pea aphid, *A. pisum*, maintained on *Vicia faba* plants in controlled conditions at 20°C, 16L/8D photoperiod, and approximately 75% RH. For experiments, aphid lines descended from a single founder were established (Kunert et al. 2005).

Experiment 1: DEBF and Aphid Behavior Three groups of 15 apterous adults of the same line were transferred to three plants and covered with cellophane bags (18.8×39 cm, *N*=12 lines). A piece of filter paper, to which DEBF, EBF (both 1 μg in 3 μl hexane), or hexane (3 μl) was applied, was placed inside the bags. The solutions were applied three times per day over 5 d. After the first application of each solution, the number of pea aphids walking was counted for 5 min. At the end of the experiment, mothers were counted and removed, and nymphs left for four more days on plants. Nymphs were then frozen and scored as winged or apterous morphs.

Experiment 2: DEBF and EBF Release For volatile collection, two groups of 15 third/fourth instars, of the same line, were transferred to two plants (*N*=13 lines) and placed in glass chambers (modified 1-L beaker). Teflon plates were placed around the base of the plant, keeping the soil out of

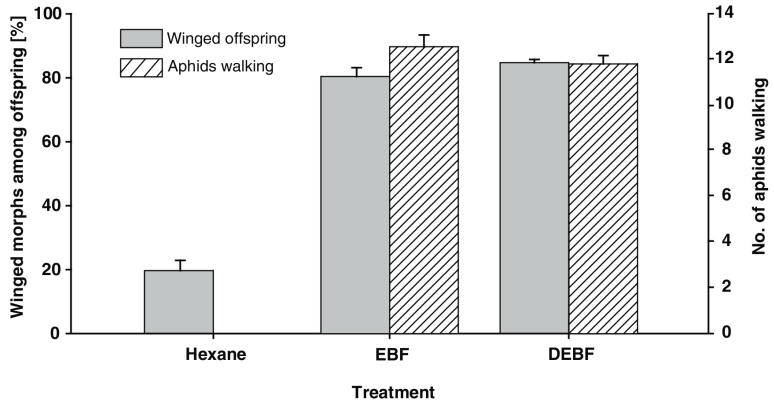

Fig. 2 Percentage of winged offspring produced by adult pea aphids and number of pea aphid mothers walking after exposure to hexane, (*E*)-β-farnesene (*EBF*) or deuterated (*E*)-β-farnesene (*DEBF*). The bars show the mean value±SE

the collection system. Two openings (Ø=1 cm) were present on top of the chamber: one provided air (2 L min^{-1}) filtered through active charcoal, and the other held a filter paper to which either DEBF (1 μg in 3 μl hexane) or hexane (3 μl) was applied. The chambers had an additional opening 3 cm from the rim holding a Super-Q filter (80/100 mesh; Alltech, Deerfield, IL, USA) connected to an air pump (1 L min^{-1}). With this set-up, six plants (three lines) could be tested simultaneously.

Volatile Analysis Super Q filters were eluted with 140 μl of dichloromethane and analyzed by GC-MS on a DB-5MS (J&W) column. For analysis, the column oven was kept at 60°C for 2 min., increased to 180°C at 5°C min^{-1}, and then increased at 60°C L min^{-1} until 300°C. Mass spectra from each peak were compared to those in the NIST and Wiley libraries for tentative peak identification. An internal standard of (*E*)-β-caryophyllene (400 ng in 30 μl of CH_2Cl_2) was added to all samples as an internal standard.

Statistical Analyses The number of aphids walking was analyzed by analysis of variance (ANOVA). Wing induction was analyzed by a generalized linear model (glm) with a quasi-binomial error structure. The aphid lines and number of nymphs produced in each replicate were included in the model, which was then simplified by removing non-significant variables or interactions, and accepted after an ANOVA ($P>0.05$; Crawley 2007). The survival of the mothers was analyzed with a glm using a Poisson error structure and simplified as described above. Data were analyzed with R software 2.6.0 (2007) and are presented as mean±SE.

Results and Discussion

In experiment 1, there was no significant difference among treatments in the mean number of adult pea aphids that survived (hexane=13.75±0.52; EBF=17.83±0.44; DEBF=13.58±0.47; $t=1.317$; $P=0.197$; $N=36$). In both EBF and DEBF treatments, the proportion of winged morphs among aphid offspring was higher than in the hexane treatment (Fig. 2; $t=15.075$; $P<0.001$, $N=36$). The number of aphids that responded to treatment by walking did not differ between EBF and DEBF (Fig. 2, $t=-1.542$, $P=0.152$, $N=36$); no walking responses were observed in the hexane treatment (Fig. 2, $t=27.211$, $P<0.001$, $N=36$).

In experiment 2, aphids started dispersing after DEBF application, but there was no measurable emission of endogenous alarm pheromone, suggesting that only attacked aphids emit EBF. Given the amounts of DEBF applied and re-collected, we estimated that the minimum amount of EBF detected by our experimental system was about 30 ng, an amount equivalent to that typically released by two to three third/fourth instars (Schwartzberg et al. 2008). Therefore, because we used colonies of 30 aphids, no more than 10% of the individuals tested, if any, could have responded by emitting their own EBF.

While signal amplification would have the advantage of alerting more aphids in the colony, it also has disadvantages. For example, it is thought that aphids may use the frequency of alarm pheromone perception as a measure of danger. Experiments with predators that induce alarm pheromone release and synthetic EBF, respectively, have shown that the proportion of winged offspring is related to the number of aphids consumed and the frequency of application of EBF (Kunert et al. 2005). Signal amplification would preclude aphids from employing the frequency of alarm pheromone release as a measure of the severity of an attack. Additionally, some natural enemies use EBF to detect their prey (Acar et al. 2001; Beale et al. 2006), and amplification of the signal would increase the danger of predator attraction.

Acknowledgments We are grateful to Ingrid Jakobi for rearing the aphids, Michael Reichelt for assisting with the GC-MS, Doreen Schachtschabel for helping with the synthesis of DEBF, Efthymia Kazantzidou for assisting with volatile collection, Emily Wheeler for valuable editorial comments, and the Max-Planck-Gesellschaft, International Max Planck Research School (IMPRS) for financial support.

References

ACAR, E. B., MEDINA, J. C., LEE, M. L., and BOOTH, G. M. 2001. Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). *Can. Entomol.* 133:3389–397.

BEALE, M. H., BIRKETT, M. A., BRUCE, T. J. A., CHAMBERLAIN, K., FIELD, L. M., HUTTLY, A. K., MARTIN, J. L., PARKER, R., PHILLIPS, A. L., PICKETT, J. A., PROSSER, I. M., SHEWRY, P. R., SMART, L. E., WADHAMS, L. J., WOODCOCK, C. M., and ZHANG, Y. H. 2006. Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proc. Natl. Acad. Sci. USA* 103:10509–10513.

CRAVOTTO, G., BALLIANO, G., ROBALDO, B., OLIARO-BOSSO, S., CHIMICHI, S., and BOCCALINI, M. 2004. Farnesyloxycoumarins, a new class of squalene-hopene cyclase inhibitors. *Bioorg. Med. Chem. Lett.* 14:1931–1934.

CRAWLEY, M. J. 2007. The R Book. p. 950. Wiley, Chichester, England.

FIELDER, S., and ROWAN, D. D. 1994. The synthesis of d(6)-alpha-farnesene. *J. Labelled Comp. Rad.* 34:1075–1085.

FRANCIS, F., VANDERMOTEN, S., VERHEGGEN, F., LOGNAY, G., and HAUBRUGE, E. 2005. Is the (E)-beta-farnesene only volatile terpenoid in aphids? *J. Appl. Entomol.* 129:16–11.

KUNERT, G., OTTO, S., ROSE, U. S. R., GERSHENZON, J., and WEISSER, W. W. 2005. Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecol. Lett.* 8:596–603.

NAULT, L. R., EDWARDS, L. J., and STYER, W. E. 1973. Aphid alarm pheromones: secretion and reception. *Environ. Entomol.* 2:101–105.

PICKETT, J. A., and GLINWOOD, R. 2007. Chemical ecology, pp. 255–260, in H.F. Van Emden, and R.H. Harrington (eds.). Aphids as Crop PestsCABI, Wallingford, Oxfordshire.

SCHWARTZBERG, E. G., KUNERT, G., STEPHAN, C., DAVID, A., RÖSE, U. S. R., GERSHENZON, J., BOLAND, W., and WEISSER, W. W. 2008. Real-time analysis of alarm pheromone emission by the pea aphid (*Acyrthosiphon pisum*) under predation. *J. Chem. Ecol.* 34:76–81.

Don't talk so loud: the emission of aphid alarm pheromone regulated by social conditions

Eduardo Hatano[1], Grit Kunert[2], Maritta Kunert[3], Anja David[3], Jonathan Gershenzon[2], Wolfgang W. Weisser[1]

[1]Institute of Ecology, Friedrich Schiller University
[2]Biochemistry Department, Max Planck Institute for Chemical Ecology
[3] Bioorganic Chemistry Department, Max Planck Institute for Chemical Ecology

In preparation for publication in *Ecological Entomology*.

ABSTRACT

Alarm signalling may have high ecological costs for the emitter, such as increase in risk of predation, because the signal can be perceived by a natural enemy. Regulation of the alarm signalling would then benefit the emitter to efficiently alarm conspecifics but at undetectable levels for the natural enemies according to surrounding conditions. Here we showed that the pea aphid *Acyrthosiphon pisum* regulates the emission of its alarm pheromone, the sesquiterpene (*E*)-β-farnesene (EBF). The analysis of amounts of EBF emitted by two aphid clones when attacked by lacewing larvae showed that isolated aphids emitted more than the amount emitted by grouped ones. However, the storage of EBF of aphids at different densities did not differ. Furthermore, the amounts of EBF emitted corresponded to a small fraction of EBF stored in the aphid body. Furthermore, the amounts emitted in both social conditions corresponded to the minimal required to trigger the wing induction in offspring showing that the regulation of EBF emission is adaptive to induce wings in offspring.

INTRODUCTION

Social interactions can affect the morphology, physiology and/or behaviour of individuals within a colony, mediating the division of labour and phenotypic plasticity, conferring the colony quick adaptations to environmental conditions (Robinson, 1992; West-Eberhard, 1989). This phenomenon is conspicuous in eusocial animals (e.g. honey bees, ants and termites) in which the colony conditions, such as colony size and phenotypes present, determines the traits of an individual. In some interactions, chemical compounds are involved, such as pheromones, and regulate and are also regulated by social interactions. For example, large colonies stimulate the emission of aggregation pheromone in desert locusts (Deng *et al.*, 1996) and social interactions alter pheromone emission in males of *Drosophila melanogaster* to estimulate females from a heterogeneous group to mate (Krupp *et al.*, 2008).

Alarm calls and group defensive strategies are also highly affected by intraspecific interactions and colony size which may reflect the risk of attack, colony stage, and level of exposure (Wilson, 2000). Aphids, for instance, are attacked by a large number of natural enemy species that differ in their mode of attack and consumption rate and, therefore, impose different threats to aphids (Völkl *et al.*, 2007). The defensive system of aphids against natural enemies includes production of soldier morphs and protection by ants in some species (Phillips & Willis, 2005; Stadler & Dixon, 2005; Stern & Foster, 1996), kicking (Chau & Mackauer, 1997; Dixon, 1958; Villagra *et al.*, 2002), and emission of fatty acids containing cornicle droplets from a pair of abdominal siphunculi. These droplets also contain an alarm pheromone which leads to walking and dropping behaviours of conspecifics to avoid the presence of a natural enemy (Bowers *et al.*, 1977; Nault *et al.*, 1973; Phelan *et al.*, 1976; Wohlers, 1980). The chemical composition of alarm pheromones was elucidated for many aphid species and usually include mainly or only the sesquiterpene (*E*)-β-farnesene (EBF) depending of species (Bowers *et al.*, 1972; Bowers *et al.*,

1977; Edwards *et al.*, 1973; Francis *et al.*, 2005; Goff & Nault, 1974; Griffiths & Pickett, 1980; Jones *et al.*, 2001; Kislow & Edwards, 1972; Nault *et al.*, 1973).

Besides the terrestrial dispersal behaviour that EBF triggers in aphid colonies, it also indirectly induces wing formation in aphid offspring (Kunert & Weisser, 2003; Sloggett & Weisser, 2002, 2004; Weisser *et al.*, 1999). This effect is induced by an increase in physical contact among alarmed aphids when they disperse (Kunert *et al.*, 2005) and simulates the tactile stimuli when aphids are in crowding conditions which also triggers the wing induction (Lees, 1967; Sutherland, 1969a).

In addition to the benefits of alarm signalling, EBF also bears costs for aphids. Because this compound is commonly used by many aphid species as alarm pheromone (Francis *et al.*, 2005), it is a reliable cue for natural enemies to find them (Vet & Dicke, 1992). Many species of ladybirds (Acar *et al.*, 2001; Al Abassi *et al.*, 2000), lacewings (Zhu *et al.*, 1999), hoverflies (Francis *et al.*, 2005), and parasitoids wasps (Micha & Wyss, 1996) perceive this cue and use it to locate aphids on a plant. Furthermore, aphids may face high mortality levels associated with the cost of stop feeding and disperse when responding to alarm pheromone, such as the risks of desiccation and starvation depending of environmental conditions (Dill *et al.*, 1990; Hatano *et al.*, 2010; Roitberg & Myers, 1978).

Therefore, it is likely that aphids control their emission of alarm pheromone depending on their surrounding to minimize these costs. According to Verheggen *et al.* (2008) and Hatano *et al.* (2008), aphids do not respond to the alarm pheromone by emitting more EBF to warn further aphids in a colony, supporting the hypothesis that aphid colonies benefit from emitting locally low amounts of EBF to remain inconspicuous to natural enemies. In addition, the propensity of aphids to emit cornicle droplets varies with their social conditions (Robertson *et al.*, 1995) and reproductive stage (Mondor *et al.*, 2000). However, few studies explored the effect of the habitat on alarm pheromone emission and correlated with ultimate consequences for aphid colonies.

Here, we examined the effect of social interactions in pheromone production and emission from pea aphids (*Acyrthosiphon pisum*) and the consequences to the colony fitness. We hypothesized that aphids regulate the amounts of EBF according to social conditions, with aphids in larger groups emitting less EBF than aphids in smaller groups, to reduce the risks associated with alarm pheromone emission while exploring its potential benefits for survival. The social conditions might affect the emission of EBF by I) affecting the stored amounts in aphids, II) affecting the aphid size which might affect the emission of EBF, or III) affecting the emission when exposing cornicle droplets. To test these mechanisms, two pea aphid clones were used since they differ in their propensity to produce winged offspring and, therefore, in their physiology. The emission of EBF was study by headspace analysis of adult aphids attacked by a lacewing larva, while the stored amounts of EBF were directly extracted from aphids. All aphids were weighted and correlated with the amounts of EBF emitted and stored. In a second experiment to study the consequences of regulating EBF emission in the aphid wing induction, we applied the previously found amounts of alarm pheromone emitted in each social condition to colonies of aphids at two frequencies of application.

MATERIALS AND METHODS

Plants and insects

The pink BP clone and the green HG clone of pea aphids were reared on a dwarf variety of broad bean, *Vicia faba* L. (variety The Sutton; Nickerson-Zwaan, UK) covered with air-permeable cellophane bags (L x W = 39 x 18.5 cm, Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland). Plants were produced in plastic pots (10 cm diameter, 8 cm high) and used for rearing aphids and for experiments when they were three-weeks old (ca. 15 cm high with 4 leaves).

Lacewing larvae, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), were purchased from Katz Biotech (www.katzbiotech.de/index.htm) and reared on plants with a mixture of BP and HG aphids until third instar when they were used for the experiments. Plants, aphids and lacewings were raised under the same controlled conditions (16:8 L:D; 20^{o}C; 75% RH).

Aphid lines

For experiments, aphid lines were established for each clone according to Kunert *et al.* (2005) to control for differences in rearing conditions among plants and maternal effects. To start an aphid line, a single adult aphid was initially placed on a three-week-old broad bean plant to reproduce for 24 hr, and then removed from the plant. After eight days, the daughters (2 aphids per line), now adults, were transferred to two new plants (1 aphid per plant). After 36 hr reproduction, the daughters were removed, leaving 6 granddaughters per plant. After 48 hr, nymphs reached the second instar and were transferred to new broad bean plants divided into two treatments (grouped and isolated) as described below. An aphid line was used as one replicate for each treatment.

Experiment 1. The effect of group-living on the emission of alarm pheromone

Density treatments

To test for the effect of group-living conditions on EBF emission, three aphids were either kept isolated during their juvenile development on different plants (isolated aphids) or grouped in the same cage (grouped aphids). Second instar nymphs were transferred to new plants and kept inside clip cages fixed to the abaxial surface of the third leaf from the bottom of a plant. Clip cages (1 cm diameter, 1.5 cm height) were made from Eppendorf tubes from which both top lid and lower end of the tube were cut off. The bottom end of the tube was closed with a muslin cloth (1 x 1 cm). Square pieces of sponge material (melamine resin, 3 x 3 cm, 0.5 cm height) with an opening in the middle (1 cm diameter) was used to hold the cages and clipped to the leaves using paper clips. From each line, three aphids were kept together in the same cage (grouped aphids) while other three aphids were kept singly in separated cages (isolated aphids) on different plants until they become adults (7 days). When aphids became adults, they were used

for the analysis of EBF emission under lacewing attack and extraction of total EBF (see below) within 24 hr after they started reproducing.

After adults were removed from the plant, their offspring produced within 24 hr were kept on the original plants without cages until they reach the adult stage, when their phenotype (winged or unwinged) was assessed to confirm the effect of crowding on aphids. The proportion of winged offspring was then determined for each clone in all treatments.

EBF emission under lacewing attack

To collect the EBF emitted, a 1.5 mg charcoal filter (5 mm diameter, Gränicher & Quatero) was connected to a hypodermic needle (0.90 x 40 mm) using a latex free disposable syringe that was cut on 1.0 ml mark, and inserted into a 4 ml borosilicate vial (L x W = 45 x 14.75 mm, Macherey & Nagel) through a Teflon septum (31 x 6 mm, Fisherbrand). The charcoal filter was then connected to an air pump with an electronic flowmeter adjustated to 0.2 ml/min. A second filter was also connected to a needle that was inserted through the septum to let clean air enter the system during collection.

Isolated and grouped aphids from 15 lines were first weighed and then individually placed into the vials together with a third instar lacewing larva (6-day old) which was also weighed. The volatile collection started immediately after the lacewing larva attacked an aphid and lasted until the predator stopped feeding which took 1.5 hr on average. For each aphid line, three aphids from the isolated aphid treatment and three from the grouped aphid treatment were analysed.

The method described by Kunert *et al.* (2009) was used to elute the charcoal filters with few modifications. Filters were connected to glass microinserts (0.1 ml, 31 x 0.6 mm, Fisherbrand) using small PTFE tubes (i.d. 4.5 mm). Twenty microliters of hexane containing β-caryophyllene (2.5 ng/µl, Sigma-Aldrich®) as internal standard were inserted in the filter and the bottom of the insert was quickly rinsed in liquid nitrogen to pull down the solvent through the charcoal layer. The bottom of insert was then warmed by hand to push solvent up and this procedure was repeated four times until the solvent was finally collected in the inlet. After that, 10 µl of the same solution was added to wash over all compounds and added to the previous aliquot. Inserts were put inside 1.5 ml borosilicate vials with a spring, closed with a septum and stored at -20^{o}C until analysis.

Total EBF stored by aphids

The total amount of EBF was extracted from aphids of 17 lines in order to study the effects of density treatments and clone type in the production of alarm pheromone. Adults were carefully removed from cages using tweezers to avoid cornicle excretion by aphids, weighed, and immersed in 20µl hexane containing 2.5 ng/µl of β-caryophyllene as internal standard. Aphids and solvent were kept in a glass inlet closed in a 1.5 ml borosilicate vial for 24 hr under -20^{o}C. Aphids were then removed from the insert and extract kept in the same condition until analysis. Preliminary experiments showed that no additional amounts of EBF can be extracted after 24 hr. For each aphid line, the amounts of EBF from three aphids from the isolated aphid treatment and three from the grouped aphid treatment were analysed.

Analysis of EBF emitted and stored

EBF eluted from charcoal filters and extracted from aphids was analysed by injecting 2 µl in a GC-MS with a Hewlett–Packard 6890 gas chromatograph equipped with a Hewlett–Packard 7683 auto sampler and a Hewlett–Packard 5973 quadrupole-type mass selective detector operated in electron impact mode. The mass detector had a transfer line temperature of 230^{o}C, a source temperature of 230^{o}C, a quadrupole temperature of 150^{o}C, electron energy of 70 eV, and a scan range of 50–400 amu. Helium was used as a carrier gas at a linear flow rate of 1 ml/min. All samples were analysed on a DB-5MS (J & W) column. After sample injection, the column oven was kept at 60^{o}C for 4 min, increased to 150 ^{o}C at a rate of 5 ^{o}C/min, and then again increased at 60^{o}C/min until 300 ^{o}C and kept for 2 min. Mass spectrum of EBF and β-caryophyllene were compared to those in the National Institute of Standards and Technology and the Wiley libraries for identification of peaks.

Experiment 2. Production of winged offspring under different EBF-crowding environments

In order to test the response to produce winged offspring to the different amounts of EBF found in the previous volatile collection, BP and HG aphids were reared in groups of two or seven aphids and exposed to different amounts of EBF solution (5 ng, 10 ng in 5 µl hexane, hexane control), applied two or five times a day. The selected amounts of EBF were the same found Experiment 1 when aphids emitted EBF.

For this experiment, six aphid lines were established for each clone as described above with few modifications. From one line, a foundress was allowed to produce nymphs for 48 hr. When offspring became adult, six daughters were separated to six new plants, kept for 48 hr on the plant to produce enough nymphs. From each line, two groups of either two or seven unwinged forth instar nymphs were put on 3-weeks-old *V. faba* and covered with cellophane bags. A piece of filter paper (1 x 1 cm, company) fixed to a wire was placed inside the bags close to the soil onto which EBF solution or hexane was applied using a glass syringe (10 µl, Hamilton). The experiment started 24 hr after aphids were transferred to plants. After five days, mothers were removed and all nymphs were left for five more days on the original plants. The offspring were then collected from each plant, frozen at -20 ^{o}C, and their phenotype was determined later.

Statistical analysis

The effects of density treatment and clone type on the amounts of EBF emitted and stored, aphid weights, number of offspring and proportion of winged morphs from clip cage experiments (Experiment 1) were analysed by Analysis of Covariance (ANCOVA). Because there were three aphids (pseudoreplicates) from each line in all treatments, amounts of EBF (volatile collection and body extraction), aphid weights and number of offspring of every three aphids were averaged at the level of lines and used as replicates for analysis. The proportions of winged offspring were calculated by summing the number of winged morphs from three aphids of a line and dividing by the respective sum of all offspring. The statistical analyses of aphid weights, number of offspring

and proportion of winged offspring used the data sets of volatile collection and body extraction combined in one data set.

In experiment 2, the proportion of winged offspring induced by synthetic EBF was calculated by dividing the number of winged morphs by the total number of offspring on a plant. The effects of EBF concentration, frequency of application and aphid density data on wing induction were analysed by a 3-way Analysis of Variance (ANOVA).

In all analyses, aphid lines were included as a random effect. Models were simplified to the minimal adequate model by stepwise removing non-significant interactions followed by independent variables that were not included in any significant interaction (Crawley, 2007b). When a variable or an interaction of variables was found to be significant, the corresponding levels were compared using treatment contrast tests (Crawley, 2007a). Data were analysed using the R software 2.9.0 (www.r-project.org) and are presented as mean $\pm$ SE.

RESULTS

Experiment 1:
Aphid weight

Weight was first natural log-transformed because of non-normality of error distribution. BP clones (3.71 $\pm$ 0.10 mg) were significantly heavier than HG clones (2.92 $\pm$ 0.07 mg, $P < 0.001$), and isolated aphids (3.47 $\pm$ 0.12 mg) were heavier than grouped ones (3.17 $\pm$ 0.08 mg, $P < 0.001$). The interaction of both significantly affected aphid weight ($P = 0.023$). Among green clones, isolated aphids were heavier than grouped ones ($t_{119} = 4.519$, $P < 0.001$, Fig. 1). However, density treatment did not significantly affect the weight of pink clones ($t_{119} = 1.341$, $P = 0.182$, Fig. 1). The statistical analysis of aphid weight is presented in Table 1A.

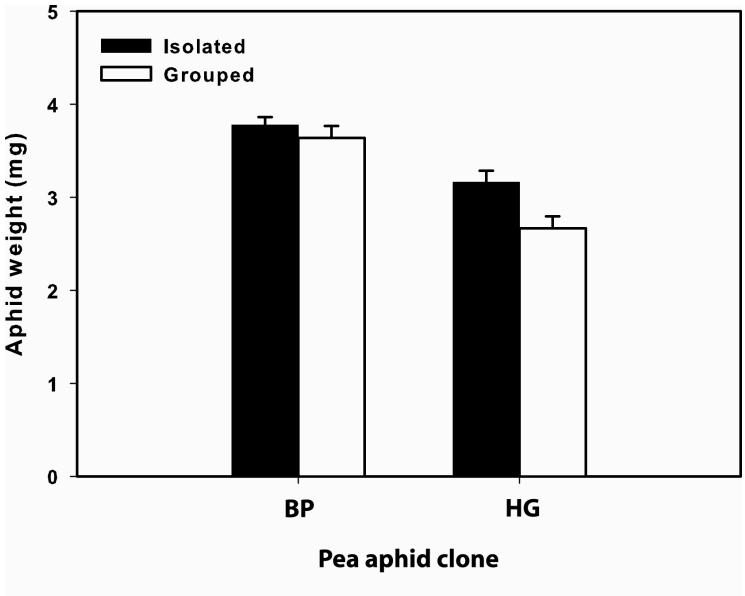

Figure 1. Body weight of BP and HG clones of pea aphids kept in isolation (black bars) or in groups (white bars). Bars represent mean + SE.

Number of offspring and proportion of winged offspring

Because the weights of BP and HG aphids were significantly affected by the density treatment, they were included in the analysis of the number of offspring and proportion of winged offspring. The total number of offspring produced in 24 hr after adult aphids started to reproduce did not differ among pea aphid clones either isolated or grouped (P = 0.137, Fig. 2, Table 1B). Aphid weight or its interactions with other variables were not significant (Table 1B).

For the analysis of proportion of winged offspring, HG clones were not included in the analysis because both isolated and grouped aphids produced only unwinged offspring. Grouped pink aphids produced a significantly higher proportion of winged offspring than isolated aphids (P < 0.001, Fig. 2, Table 1C). Aphid weight and its interaction with density treatment were not significant (Table 1C).

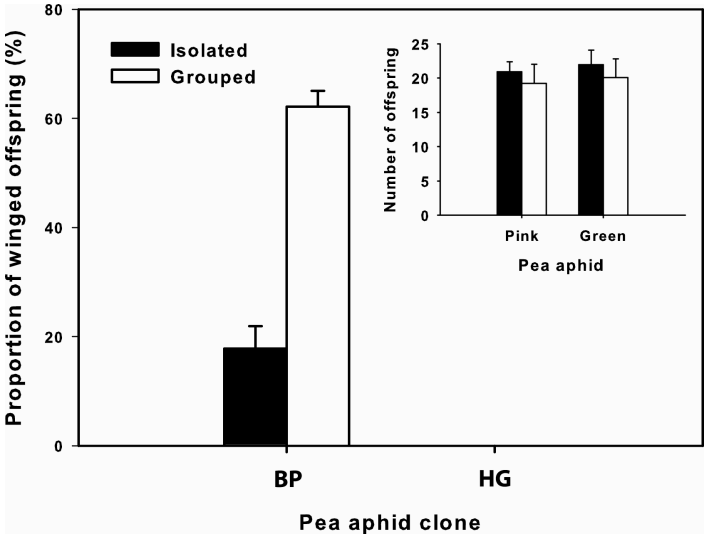

Figure 2. Proportion of winged offspring and total number of offspring from BP and HG clones of pea aphids kept in isolation (black bars) or in groups (white bars). Bars represent mean values + SE.

EBF stored and EBF emitted

Previous to analysis, the amounts of EBF extracted from aphids were natural log transformed because of non-normality of error distribution. Aphids of the BP clone stored more EBF than aphids from the HG clone (P < 0.05, Fig. 3A), but the amount of EBF per aphid was not affected by the density treatment (P = 0.211, Fig. 3A). The amounts of stored EBF differed among aphid lines (P < 0.001) and had a negative relation with their weight (P = 0.049). The analysis of the amounts of EBF stored is presented in Table 2A.

There was no difference in the amount of EBF emitted by aphids from the BP and HG clone (P = 0.329), but isolated aphids emitted on average 98% more EBF than grouped aphids when attacked by third instar lacewing larvae (P < 0.05, Fig. 3B). The amounts of EBF emitted from isolated and grouped aphids, however, did not resemble the amounts of EBF stored. Aphids of isolated and grouped treatments released on average only 9.2% and 4.7% of the respective

stored amounts and were significantly different (t_{56} = 2.412, *P* = 0.019). The amounts of EBF emitted were independent of the aphid weight (*P* = 0.878), lacewing weight (*P* = 0.899) and the interaction between clone type and density treatment (*P* = 0.287). The analysis of the amounts of EBF emitted is presented in Table 2B.

Table 1. Analyses of aphid weight (A), number of offspring (B), proportion of winged offspring (C).

Experiment	Source of variation	df	χ^2	*F*	*P*	
A. Aphid weight[1]						
	Clone (Cl)	1	18.91	75.44	<0.001	***
	Treatment (Tr)	1	2.93	16.93	<0.001	***
	Line	61	0.24	0.95	0.580	
	Cl:Tr	1	0.99	5.28	0.023	*
	Residual	53	1.04			
B. Number of offspring						
	Clone (Cl)	1	27.80	0.63	0.431	
	Treatment (Tr)	1	97.20	2.09	0.152	
	Aphid weight (Aph)	1	23.2	0.53	0.471	
	Line	61	86.80	1.86	0.011	*
	Cl:Tr	1	4.00	0.09	0.769	
	Cl:Aw	1	0.20	0.00	0.948	
	Tr:Aw	1	17.3	0.39	0.536	
	Cl:Tr:Aw	1	20.80	0.44	0.508	
	Residual	53	2477.6			
C. Proportion of winged offspring (BP clone)						
	Treatment (Tr)	1	3.09	120.42	<0.001	***
	Aphid weight (Aw)	1	0.02	1.02	0.319	
	Line	31	0.95	1.52	0.127	
	Tr:Aw	1	0.00	0.03	0.854	
	Residuals	28	0.57			

[1] Aphids weights were natural log transformed.

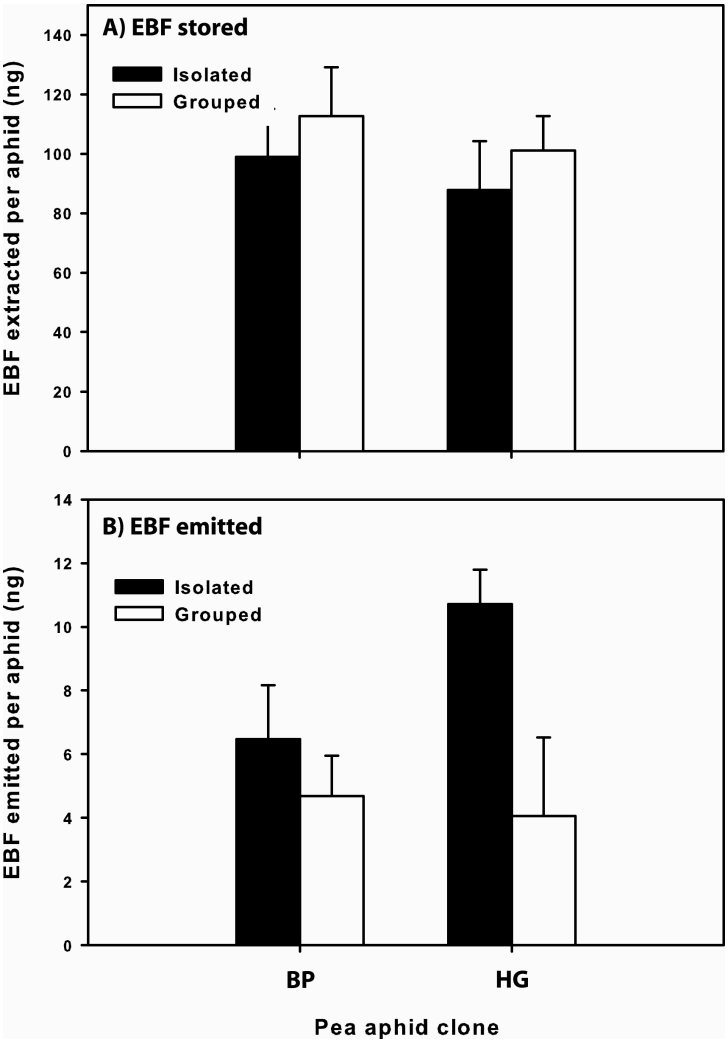

Figure 3. Amounts (ng) of EBF stored (A), and emitted when attacked by lacewing larvae (B) from BP and HG clones of pea aphid kept in isolation (black bars) or grouped (white bars). Bars represent mean + SE.

Table 2. Analysis of amount of EBF extracted (A) and EBF emitted from aphids when attacked by lacewings larvae (B).

Experiment	Source of variation	df	χ^2	*F*	*P*	
A. EBF stored[1]						
	Clone (Cl)	1	0.40	6.26	0.018	*
	Treatment (Tr)	1	0.11	1.80	0.189	
	Aphid weight (Aw)	1	0.27	4.22	0.049	*
	Line	31	0.26	4.03	<0.001	***
	Cl:Tr	1	0.01	0.14	0.711	
	Cl:Aw	1	0.05	0.78	0.385	
	Tr:Aw	1	0.02	0.37	0.550	
	Cl:Tr:Aw	1	0.06	0.80	0.380	
	Residual	25	1.67			
B. EBF emitted						
	Clone (Cl)	1	0.01	1.17	0.285	
	Treatment (Tr)	1	0.05	4.95	0.043	*
	Aphid weight (Aw)	1	0.00	0.02	0.869	
	Lacewing weight (Lw)	1	0.00	0.02	0.893	
	Line	28	0.23	0.96	0.547	
	Cl:Tr	1	0.01	1.19	0.287	
	Cl:Aw	1	0.02	2.41	0.135	
	Tr:Aw	1	0.01	1.31	0.265	
	Cl:Lw	1	0.00	0.03	0.857	
	Tr:Lw	1	0.00	0.33	0.569	
	Aw:Lw	1	0.02	2.21	0.153	
	Cl:Tr:Aw	1	0.00	0.12	0.738	
	Cl:Tr:Lw	1	0.01	0.77	0.391	
	Cl:Aw:Lw	1	0.00	0.11	0.746	
	Tr:Aw:Lw	1	0.02	1.76	0.205	
	Cl:Tr:Aw:Lw	1	0.00	0.07	0.793	
	Residual	14	0.13			

[1] Amounts of EBF extracted from aphids were natural log transformed.

Experiment 2. Production of winged offspring under different EBF-crowding environments

Green pea aphids did not produce any winged offspring and were not included in the statistical analysis. Pink pea aphids, on the contrary, were very responsive to alarm pheromone in producing winged offspring (Fig. 4). Statistical analysis of this experiment is presented in Table 3.

Colony size had a significant effect with 2 aphids-colonies and 7 aphid-colonies producing 1.78 ± 0.37% and 2.53 ± 0.47% winged offspring, respectively (t = 3.76, $P < 0.05$, Fig. 4). The interaction between the EBF concentration and frequency of application was significant ($P < 0.05$, Fig. 4). When EBF concentrations were applied two times per day, 10 ng EBF solution significantly increased the proportion of winged offspring compared to the control (t = 9.53, $P < 0.05$, Fig. 4), but not the application of 5 ng EBF (t = 12.50, $P = 0.637$, Fig. 4). When solutions were applied five times per day, 5 ng and 10 ng EBF significantly increased the proportion of winged morphs comparing to the control (t = 9.936, $P < 0.05$, and t = 7.797, $P < 0.001$, respectively, Fig. 4). The interaction among colony size, frequency of application and EBF concentration was not significant ($P = 0.203$).

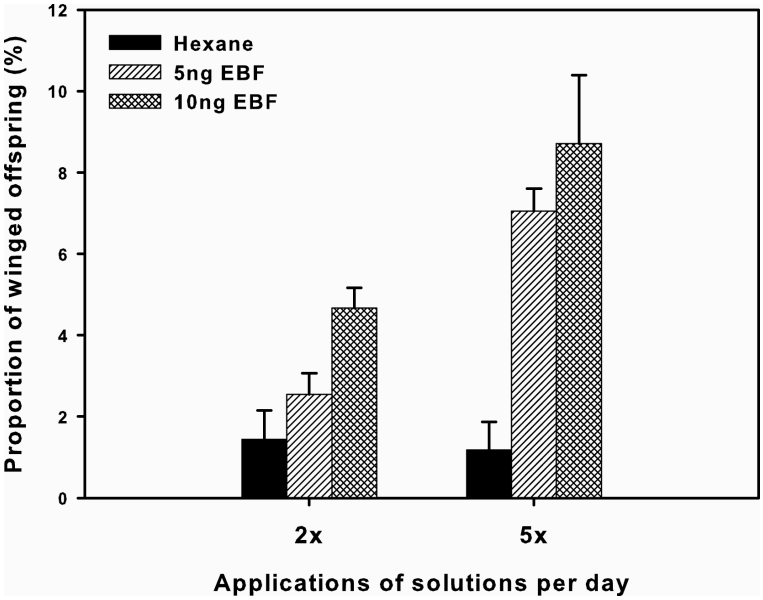

Figure 4. Proportion of winged offspring from BP pea aphids treated with EBF applied two or five times per day. Bars represent mean ± SE.

Table 3. Analysis of proportion of winged offspring from aphids of different colony sizes exposed to different frequencies and amounts of EBF.

Experiment	Source of variation	DF	χ^2	F	P	
Proportion of winged offspring (BP)						
	EBF concentration (Cn)	2	0,02	20,62	<0.001	***
	Frequency (Fr)	1	0,01	16,28	<0.001	***
	Colony size (Cs)	1	0,00	4,47	0,038	*
	Line	5	0,00	1,16	0,340	
	Cn:Fr	2	0,00	4,93	0,010	**
	Cn:Cs	2	0,00	1,92	0,157	
	Fr:Cs	1	0,00	0,78	0,380	
	Cn:Fr:Cs	2	0,00	1,67	0,197	
	Residuals	55	0,00			

DISCUSSION

The results of our experiments clearly demonstrate that the emission of alarm pheromone is regulated by the colony size. Interestingly, the amounts of EBF emitted by pea aphids were only small fractions of the stored amounts. Although the BP clone and HG clone belongs to the same species, there was a significant variation between them in response to group size: while HG clone produced no winged offspring in both density treatments, the BP clone, which was reported previously as being very sensitive to physical contact (Kunert *et al.*, 2005; Sloggett & Weisser, 2002), readily produced a higher proportion of winged offspring in the group treatment. In addition, grouping did not influence the number of offspring in either clone, but negatively affected body weight in the HG clone only. Thus, although both clones responded differently to crowding conditions, both perceived the social environment surrounding them.

We then tested the hypothesis that grouped aphids emit less amounts of alarm pheromone than isolated aphids to alert conspecifics while remaining inconspicuous to natural enemies. The emission of alarm pheromone was strongly determined by the social environment and grouped aphids emitted less amounts of EBF than isolated aphids. The amounts of EBF emitted was independent of the aphid body weight, suggesting that indirect effects, such as nutritional constraints, were unlikely involved. In addition, the amounts of EBF emitted by BP and HG aphids did not differ either. One possible explanation for the decrease in EBF emitted by grouped aphids is that aphids respond to the distribution of individuals on a plant. As aphids are rarely single on a plant, the physical contact between individuals may indicate that conspecifics are in close proximity, so emitting low amounts of EBF will warn these aphids, but will still keep the signal less inconspicuous to natural enemies. In contrast, the higher amounts emitted by isolated aphids

would warn aphids further away that are not in close range and in physical contact. Since, when attacked by a natural enemy, the chances of such small groups of aphids to survive is lower than larger groups, the benefits of emitting higher amounts of EBF to alarm conspecifics in small groups would increase the survival rates even under the risk of attracting other natural enemies. For instance, similar strategy is observed in the social wasp, *Polybia occidentalis*, which invests more attacks when coming from small nests than individuals from larger nests and is positively correlated with its investment in brood (London & Jeanne, 2003).

Conversely to the emission of EBF, the storage of EBF was not affected by the group conditions, suggesting that either aphids do not have a regulatory system for producing EBF or the production of EBF is not expensive for pea aphids. Mondor and Roitberg (2003) demonstrated that fitness cost of emitting cornicle droplet is age-dependent, but adult pea aphids that emitted droplets did not reduce their reproductive fitness and intrinsic rate of increase, enforcing the hypothesis that the production of EBF may be energetically cheap for adult pea aphids. In addition, the amounts of EBF stored in pea aphids were approximately 10 and 24 times the amounts emitted by isolated and grouped aphids respectively. Although the ecological relevance of such super production of EBF is still unknown, one possible explanation is that aphids store high amounts of EBF to emit a second cornicle droplet in case they evade the first predator and are attacked again in a short interval (Strong, 1967; Wynn & Boudreau, 1972). Furthermore, it is not ecologically advantageous for aphids to emit conspicuous amounts of EBF that can be perceived by natural enemies and used as a kairomone to locate the source.

Verheggen *et al.* (2009) also studied the emission of EBF from *A. pisum* at different social environments and interestingly demonstrated the converse: aphids grouped with conspecifics or *Myzus persicae* emitted more pheromone than isolated ones, and this regulation is mediated by volatile cues from the group, probably EBF. Differences in the regulation of EBF emission observed in Verheggen *et al.*'s work and our results might come from the physiological adult state of aphids tested: while adults at the prereproductive stage were used in Verheggen *et al.* (2009), here we used aphids that were reproducing for 24 hr (reproductive stage). Pea aphids vary the amounts of EBF emitted according to different stages, with the highest amount emitted in the third instar and decreasing to adult stage (Mondor *et al.*, 2000). However, we lack the information whether amounts of EBF emitted vary among adults of different ages. Another possible reason is that since aphids were crushed to emit EBF in Verheggen's work, aphids might respond differently to a natural attack. There is a variation in the amount of EBF emitted by pea aphids according to type of predatory manipulation. For instance, the feeding of *Aphidoletes aphidimyza* and *Leucopis annulipes*, which are furtive predators and do not trigger the escape behaviour of aphids (Frechette *et al.*, 2008; Lucas & Brodeur, 2001), comparing with ladybirds and lacewings which elicits the alarm signalling, probably because of their disruptive feeding behaviour (Dixon, 1998). No study, however, has compared the amounts of EBF emitted when an aphid is artificially damaged and attacked by a natural enemy. Finally, in the work of Verheggen *et al.* (2009) aphids fed on an artificial diet, while here we used 3-week old broad bean plants, and this difference in food source might also have influenced both experiments. Therefore there are other potential factors, such as predator type, physiological age and food source, acting additionally or

synergistically with social environments that determine the regulation of EBF emission in pea aphids.

Kunert *et al.* (2005) provided evidence that the amount of EBF applied to an aphid colony is not as crucial as the frequencies of alarm signalling to determine the morphology of offspring. The induction of wings is not directly influenced by the EBF, but is triggered by the increased physical contact among aphids when they are alarmed by EBF (Kunert *et al.*, 2005). Here, we examined the effect of the amounts of EBF emitted by isolated and grouped aphids (10 ng and 5 ng, respectively) and also found a significant interaction between the amounts EBF applied and frequency of application to induce wing formation which was independent of colony size. However, a comparison within the two times application frequency showed that 5 ng EBF did not significantly increase the proportion of winged morphs compared to control, while 10 ng EBF did. Within the five times application frequency, both 5 ng and 10 ng significantly increased the production of winged morphs. It suggests that at low population densities, the high amount of EBF emitted by isolated aphids (i.e. 10 ng) can trigger the dispersal system in pink pea aphid colonies, possibly because the higher amounts are likely to be perceived by aphids and trigger the dispersal behaviour. Therefore, low amounts of EBF (i.e. 5 ng) emitted by grouped aphids are enough to trigger the dispersal system of a colony at high densities. It is likely then that the amounts of alarm pheromone determined by the social conditions are enough to stimulate the wing induction.

Unlike the BP clone, the HG clone did not produce winged offspring when EBF was applied. In general, green pea aphids are usually less responsive in producing winged morphs than pink ones when crowded (Sutherland, 1969a), or when predators are present (Schwartzberg *et al.*, 2008; Weisser *et al.*, 1999), but the proportion of winged morphs can increase when a low nutritional host is offered after tactile stimulus (Sutherland, 1967, 1969b). Since a few winged individuals of the HG clone were observed in our stock colonies only at very high population densities on senescing plants, it is likely that our application of EBF and also the group condition in clip cages of Experiment 1 did not reach the threshold to trigger wing induction in HG clones. Possibly, the different degrees of wing induction among pea aphids are related to genetic variation shaped by local top-down and bottom-up pressures, since they also influence the distribution genetic diversity of pea aphids in natural ecosystems

We have shown that pea aphids regulate their emission of alarm pheromone according to their colony size: aphids in groups emit less amounts of EBF than isolated aphids. This emission was not correlated with the amounts of EBF stored in the aphid body or body weight, suggesting that the emission is regulated when pea aphids emit the cornicle droplet. This regulation would then allow aphids to quickly modify their alarm signalling to different situations to enhance the fitness of the colony. Furthermore, the amounts emitted were still enough to trigger the induction of wings in colonies of different sizes and likely maintain the colony undetectable to natural enemies. Therefore, because the emission of this alarm pheromone also represents costs, intraspecific interactions in the group alleviate this selective pressure by influencing the individual fitness according to the colony needs.

ACKNOWLEDGEMENTS

We wish to thank Judith Pell and Jason Baverstock from Rothamsted Research for kindly providing the HG clone, Katz Biotech for providing lacewing larvae, and the International Max Planck Research School in Jena for financial support.

REFERENCES

Acar, E.B., Medina, J.C., Lee, M.L., & Booth, G.M. (2001) Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). Can. Entomol., 133, 389-397.

Al Abassi, S., Birkett, M.A., Pettersson, J., Pickett, J.A., Wadhams, L.J., & Woodcock, C.M. (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. J. Chem. Ecol., 26, 1765-1771.

Bowers, W.S., Nault, L.R., Webb, R.E., & Dutky, S.R. (1972) Aphid alarm pheromone: isolation, identification, synthesis. Science, 177, 1121-1122.

Bowers, W.S., Nishino, C., Montgomery, M.E., & Nault, L.R. (1977) Structure-activity-relationships of analogs of aphid alarm pheromone, (*E*)-beta-farnesene. J. Insect Physiol., 23, 697-701.

Chau, A. & Mackauer, M. (1997) Dropping of pea aphids from feeding site: A consequence of parasitism by the wasp, *Monoctonus paulensis*. Entomol. Exp. Appl., 83, 247-252.

Crawley, M.J. (2007a). Classical tests. In The R book (ed M.J. Crawley), pp. 279-322. John Wiley & Sons Ltc, Chichester.

Crawley, M.J. (2007b). Statistical modelling. In The R book (ed M.J. Crawley), pp. 323-386. John Wiley & Sons Ltd, Chichester.

Deng, A.L., Torto, B., Hassanali, A., & Ali, E.E. (1996) Effects of shifting to crowded or solitary conditions on pheromone release and morphometrics of the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). J. Insect Physiol., 42, 771-776.

Dill, L.M., Fraser, A.H.G., & Roitberg, B.D. (1990) The economics of escape behavior in the pea aphid, *Acrythosiphon pisum*. Oecologia, 83, 473-478.

Dixon, A.F.G. (1958) The escape responses shown by certain aphids to the presence of the coccinelid *Adalia decempunctata* (L.). Trans. R. Ent. Soc. London, 110, 319-334.

Dixon, A.F.G. (1998) Aphid ecology, Second edition edn. Chapman & Hall, London.

Edwards, L.J., Siddall, J.B., Dunham, L.L., Uden, P., & Kislow, C.J. (1973) Trans-beta-farnesene, alarm pheromone of the green peach aphid, *Myzus persicae* (Sulzer). Nature, 241, 126-127.

Francis, F., Martin, T., Lognay, G., & Haubruge, E. (2005) Role of (*E*)-beta-farnesene in systematic aphid prey location by *Episyrphus balteatus l*arvae (Diptera: Syrphidae). Eur. J. Entomol., 102, 431-436.

Frechette, B., Larouche, F., & Lucas, E. (2008) *Leucopis annulipes* larvae (Diptera : Chamaemyiidae) use a furtive predation strategy within aphid colonies. Eur. J. Entomol., 105, 399-403.

Goff, A.M. & Nault, L.R. (1974) Aphid cornicle secretions ineffective against attack by parasitoid wasps. Environ. Entomol., 3, 565-566.

Griffiths, D.C. & Pickett, J.A. (1980) A potential application of aphid alarm pheromones. Entomol. Exp. Appl., 27, 199-201.

Hatano, E., Kunert, G., Bartram, S., Boland, W., Gershenzon, J., & Weisser, W.W. (2008) Do aphid colonies amplify their emission of alarm pheromone? J. Chem. Ecol., 34, 1149-1152.

Hatano, E., Kunert, G., & Weisser, W.W. (2010) Aphid wing induction and ecological costs of alarm pheromone emission under field conditions. PLoS One. Accepted.

Jones, A.M.E., Bridges, M., Bones, A.M., Cole, R., & Rossiter, J.T. (2001) Purification and characterisation of a non-plant myrosinase from the cabbage aphid *Brevicoryne brassicae* (L.). Insect Biochem. Molec. , 31, 1-5.

Kislow, C. & Edwards, L.J. (1972) Repellent odour in aphids. Nature, 235, 108-109.

Krupp, J.J., Kent, C., Billeter, J.C., Azanchi, R., So, A.K.C., Schonfeld, J.A., Smith, B.P., Lucas, C., & Levine, J.D. (2008) Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. Curr. Biol., 18, 1373-1383.

Kunert, G., Otto, S., Rose, U.S.R., Gershenzon, J., & Weisser, W.W. (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecol. Lett., 8, 596-603.

Kunert, G. & Weisser, W.W. (2003) The interplay between density-and trait-mediated effects in predator-prey interactions: a case study in aphid wing polymorphism. Oecologia, 135, 304-312.

Kunert, M., David, A., Becher, J., & Boland, W. (2009) Volatile sampling from biological sources by the Closed-Loop-Stripping Technique. CSH Protocols, pdb.prot5233.

Lees, A.D. (1967) Production of apterous and alate forms in aphid *Megoura viciae* Buckton with special reference to role of crowding. J. Insect Physiol., 13, 289-318.

London, K.B. & Jeanne, R.L. (2003) Effects of colony size and stage of development on defense response by the swarm-founding wasp *Polybia occidentalis*. Behav. Ecol. Sociobiol., 54, 539-546.

Lucas, E. & Brodeur, J. (2001) A fox in sheep's clothing: Furtive predators benefit from the communal defense of their prey. Ecology, 82, 3246-3250.

Micha, S.G. & Wyss, U. (1996) Aphid alarm pheromone (*E*)-ß-farnesene: a host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). Chemoecology, 7, 132-139.

Mondor, E.B., Baird, D.S., Slessor, K.N., & Roitberg, B.D. (2000) Ontogeny of alarm pheromone secretion in pea aphid, *Acyrthosiphon pisum*. J. Chem. Ecol., 26, 2875-2882.

Mondor, E.B. & Roitberg, B.D. (2003) Age-dependent fitness costs of alarm signaling in aphids. Can. J. Zool., 81, 757-762.

Nault, L.R., Edwards, L.J., & Styer, W.E. (1973) Aphid alarm pheromones: secretion and reception. Environ. Entomol., 2, 101-105.

Phelan, P.L., Montgomery, M.E., & Nault, L.R. (1976) Orientation and locomotion of apterous aphids dislodged from their hosts by alarm pheromone. Ann. Entomol. Soc. Am. , 69, 1153-1156.

Phillips, I.D. & Willis, C.K.R. (2005) Defensive behavior of ants in a mutualistic relationship with aphids. Behav. Ecol. Sociobiol., 59, 321-325.

Robertson, I.C., Roitberg, B.D., Williamson, I., & Senger, S.E. (1995) Contextual chemical ecology: An evolutionary approach to the chemical ecology of insects. Am. Entomol., 41, 237-239.

Robinson, G.E. (1992) Regulation of division-of-labor in insect societies. Annu. Rev. Entomol., 37, 637-665.

Roitberg, B.D. & Myers, J.H. (1978) Adaptation of alarm pheromone responses of pea aphid *Acyrthosiphon pisum* (Harris). Can. J. Zool., 56, 103-108.

Schwartzberg, E.G., Kunert, G., Roese, U.S.R., Gershenzon, J., & Weisser, W.W. (2008) Alarm pheromone emission by pea aphid, *Acyrthosiphon pisum*, clones under predation by lacewing larvae. Entomol. Exp. Appl., 128, 403-409.

Sloggett, J.J. & Weisser, W.W. (2002) Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*. Oikos, 98, 323-333.

Sloggett, J.J. & Weisser, W.W. (2004) A general mechanism for predator- and parasitoid-induced dispersal in the pea aphid, *Acyrthosiphon pisum* (Harris). In 6th International Symposium on Aphids (eds J.-C. Simon, C.A. Dedryver, C. Rispe & M. Hullé), pp. 79-85, Institut National de la Recherche Agronomique, Rennes, France.

Stadler, B. & Dixon, A.F.G. (2005) Ecology and evolution of aphid-ant interactions. Annu. Rev. Ecol. Evolution Syst., 36, 345-372.

Stern, D.L. & Foster, W.A. (1996) The evolution of soldiers in aphids. Biol. Rev. Cambridge Philosophic. Soc., 71, 27-79.

Strong, F.E. (1967) Observations on aphid cornicle secretions. Ann. Entomol. Soc. Am., 60, 668-673.

Sutherland, O.R.W. (1967) Role of host plant in production of winged forms by a green strain of pea aphid *Acyrthosiphon pisum* Harris. Nature, 216, 387-388.

Sutherland, O.R.W. (1969a) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol., 15, 1385-1410.

Sutherland, O.R.W. (1969b) The role of host plant in production of winged forms by two strains of pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol., 15, 2179-2201.

Verheggen, F.J., Haubruge, E., De Moraes, C.M., & Mescher, M.C. (2009) Social enviroment influences aphid production of alarm pheromone. Behav. Ecol., 20, 283-288.

Verheggen, F.J., Mescher, M.C., Haubruge, E., De Moraes, C.M., & Schwartzberg, E.G. (2008) Emission of alarm pheromone in aphids: A non-contagious phenomenon. J. Chem. Ecol., 34, 1146-1148.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol., 37, 141-172.

Villagra, C.A., Ramirez, C.C., & Niemeyer, H.M. (2002) Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. Anim. Behav., 64, 677-683.

Völkl, W., Mackauer, M., Pell, J.K., & Brodeur, J. (2007). Predators, parasitoids and pathogens. In Aphid as crop pests (eds H.F.v. Emden & R. Harrington), pp. 187-234. CAB International, Wallingford.

Weisser, W.W., Braendle, C., & Minoretti, N. (1999) Predator-induced morphological shift in the pea aphid. P. Roy. Soc. London B, 266, 1175-1181.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst., 20, 249-278.

Wilson, E.O. (2000). Group selection and altruism. In Sociobiology: The new synthesis (ed E.O. Wilson), pp. 106-129. Belknap Press, Cambridge.

Wohlers, P. (1980) Escape responses of pea aphids, *Acyrthosiphon pisum* to alarm pheromones and additional stimuli. Entomol. Exp. Appl., 27, 156-168.

Wynn, G.G. & Boudreau, H.B. (1972) Structure and function of aphid cornicles - Homoptera-Aphididae. Ann. Entomol. Soc. Am., 65, 157-166.

Zhu, J.W., Cosse, A.A., Obrycki, J.J., Boo, K.S., & Baker, T.C. (1999) Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: Electroantennogram and behavioral responses. J. Chem. Ecol., 25, 1163-1177.

Entomopathogenic fungi stimulate transgenerational wing induction in pea aphids, *Acyrthosiphon pisum* (Homoptera: Aphididae)

Eduardo Hatano[1], Jason Baverstock[2], Grit Kunert[3], Judith Pell[2], Wolfgang W. Weisser[1]

[1]Institute of Ecology, Friedrich Schiller-University
[2]Biochemistry Department, Max Planck Institute for Chemical Ecology
[3] Plant and Invertebrate Ecology Division, Rothamsted Research

In preparation for publication in the *Journal of Invertebrate Pathology*.

ABSTRACT

Aphid natural enemies include not only predators and parasitoids but also pathogens, of which fungi are the most studied. Although arthropod natural enemies and adverse abiotic conditions affect wing induction in aphids, the effect of pathogens on this phenomenon has not been assessed. Here we studied the effect of two entomopathogenic fungi, *Pandora neoaphidis* and *Beauveria bassiana*, an aphid specialist and a generalist entomopathogen respectively, on maternally induced wing formation in offspring of the pea aphid, *Acyrthosiphon pisum*. We first demonstrate that pea aphids infected with either pathogen and maintained in groups on broad bean plants produced a higher proportion of winged morphs than uninfected control aphids. We then show that, when maintained in isolation, aphids infected with either pathogen also produced higher proportions of winged offspring than control aphids. There was no difference between *P. neoaphidis* and *B. bassiana* in their effects on wing induction in either experiment. Unlike the effect of predators and parasitoids on pea aphid wing induction, the effect of pathogens is independent of physical contact with other aphids, suggesting that they directly affect the physiology of the host aphid. It is possible that aphids benefit from wing induction by escaping infected patches whilst pathogens may benefit through dispersion within hosts. Possible mechanisms of wing induction are discussed.

INTRODUCTION

Polyphenism describes the environmentally-induced morphological traits of an individual that allows the development of new features whilst preserving the established ones (West-Eberhard, 1989). Among insects, aphids have conspicuous annual phenotypic plasticity. Their annual life cycle consists of sexual morphs, in which one or both sexes will have wings during autumn (depending on species) and exclusively parthenogenetically reproducing females during summer (Dixon, 1998a; Minks & Harrewijn, 1987; Williams & Dixon, 2007). Although parthenogenetic offspring carry the same genotype of their mothers, they can be diverse in their wing phenotype depending on biotic and abiotic stimuli (Dixon, 1998b; Minks & Harrewijn, 1987; Williams & Dixon, 2007). Wing induction varies among aphid species (Kunert *et al.*, 2008; Mondor *et al.*, 2005) and within clones of a species (Schwartzberg *et al.*, 2008; Sutherland, 1969a, 1969b; Weisser & Braendle, 2001; Weisser *et al.*, 1999).

Aphids may trigger wing formation directly during their nymphal stage or maternally under adverse conditions. *Aphis fabae* Scopoli and *Sitobion avenae* (F.) are examples of the first case where crowding by surrounding aphids triggers a post-natal wing formation response in early instars (Ankersmit & Dijkman, 1983; Shaw, 1970b). Other species, such as the pea aphid, *Acyrthosiphon pisum* (Harris), have wing formation triggered exclusively by maternal effects. In *A. pisum*, mothers that are submitted to crowding conditions trigger wing formation in their offspring. However, crowding is not the only factor that affects wing induction; host nutrition (Johnson, 1966; Lees, 1967; Sutherland, 1967), temperature (Hodgson *et al.*, 2005; Johnson, 1966; Lees,

1967) and presence of predators (Dixon & Agarwala, 1999; Kunert & Weisser, 2003; Weisser *et al.*, 1999) and parasitoids (Sloggett & Weisser, 2002, , 2004) can also induce wing formation in offspring. Wing induction in pea aphids triggered by arthropod natural enemies is indirectly mediated by alarm pheromone, which is emitted when aphids are attacked. When aphids detect alarm pheromone, they withdraw their stylet and walk or drop from the plant. This movement increases the encounter rate with other aphids and simulates constant physical contact, thus mimicking crowded conditions and triggering wing induction (Kunert *et al.*, 2005; Podjasek *et al.*, 2005). Therefore, wing induction in pea aphids is dependent not only on the alarm pheromone but also on the presence of additional aphids to maintain the physical contact.

Whilst the effect of arthropod natural enemies on the ecology of aphids, including their biological control, is well studied, pathogens are less frequently investigated (Roy & Cottrell, 2008). *Pandora neoaphidis* (Remaudiére and Hennebert) Humber (Zygomycota: Entomophthorales) is a specialist pathogenic fungus of aphids causing epizootics in aphid populations in temperate regions (Pell *et al.*, 2001). Conidia of this fungus are dispersed on wind currents (Hemmati *et al.*, 2001; Wilding & Perry, 1980) and on arthropod natural enemies (Baverstock *et al.*, 2009; Roy & Pell, 2000; Roy *et al.*, 1998). In the latter case, conidia can attach and form secondary conidia on the surface of non-target insects which subsequently vector the fungus to previously uninfected aphid populations (Pell *et al.*, 1997; Roy *et al.*, 2001; Roy *et al.*, 1998). In addition, natural enemies can indirectly increase infection levels by alarming aphids and enhancing the probability of making contact with conidia deposited on the plant surface (Baverstock *et al.*, 2008a; Roy *et al.*, 1998). Once attached to the aphid, conidia germinate and penetrate the cuticle and initiate the infection process (Völkl *et al.*, 2007). *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a generalist entomopathogenic fungus which is able to infect many insect species from different orders (Vega *et al*., 2009) Like *P. neoaphidis*, conidia of *B. bassiana* can be aerially dispersed (Shimazu *et al.*, 2002) or vectored by arthropod natural enemies, however, in the latter the conidia may germinate and infect the vector (Meyling *et al.*, 2006). Although these two pathogens affect the number, but not the reproductive potential, of nymphs produced by infected pea aphids, the effect on the morphotype of offspring is still unexplored.

Here we evaluated the effect of the aphid-specific pathogen, *P. neoaphidis*, and the generalist pathogen, *B. bassiana*, on maternally induced wing formation in aphid offspring. We hypothesized that I) entomopathogenic fungi induce wing formation in aphid offspring, II) entomopathogenic fungi-induced wing formation occurs through a direct process (as opposed to indirectly via response to alarm pheromone), and III) the co-evolved aphid-specific pathogen, *P. neoaphidis*, has a stronger effect than the generalist *B. bassiana*. For this purpose, we determined the effect of pathogen species and number of offspring on the proportion of winged offspring. In order to investigate whether the pseudo crowding effect is necessary to induce wing formation, aphids were inoculated either in isolation or in groups and their offspring compared.

MATERIALS AND METHODS

Aphids and pathogens

Pink pea aphids of clone BP were reared on three-week-old broad bean plants, *Vicia faba* (L.) (cultivar The Sutton; Nickerson-Zwaan, UK) in cages and maintained at 18°C and 16:8 L:D. This was the same clone that was studied for the effect of *Coccinella septempunctata* (L.) and *Aphidius ervi* (Haliday) on wing induction (Sloggett & Weisser, 2002; Weisser *et al.*, 1999) and its mechanism via alarm pheromone (Kunert *et al.*, 2005).

Pandora neoaphidis isolate X4, from the Rothamsted Research collection, was maintained as an *in vivo* culture by regular passage through *A. pisum* (Wilding, 1970). Dried *P. neoaphidis*-infected cadavers were stored at 4 °C and 20% relative humidity in darkness until required and for no longer than 6 months. For experiments, three dried cadavers were put on the surface of agar (1.5%) in a Petri dish (5 cm i.d.) and kept inside a plastic box at 10 °C and >95% relative humidity for 18h in darkness to initiate sporulation (Baverstock *et al.*, 2005).

Beauveria bassiana (Mycotech strain GHA) was stored at -86 °C in cryovials with 10% glycerol as cryoprotectant. Prior to experiments, the fungus was defrosted, streaked onto the surface of Sabouraud Dextrose Agar (SDA) plates and placed at 25 °C in darkness. After 2 weeks, the fungus had grown sufficiently for conidia to be harvested and used for experiments.

Experiment 1: Grouped aphids

Aphid lines

Fifteen aphid lines were established for this experiment according to Kunert *et al.* (2005) with few modifications. Each line provided aphids which were used as one replicate per treatment. Prior to experiments, fifteen wingless adult aphids (f_0 generation) were randomly selected and kept individually on three-week-old broad bean plants. Plants were covered with a cellophane bag (30 x 20 x 10 cm, Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) and maintained in a cabinet at 18 °C; 16:8 L:D. After 48 hours, adult aphids were removed and their progeny (f_1 generation) were left on plants for a further ten days under the same conditions until they became adults. Eight adult aphids from each plant were then transferred, individually, to new 3-weeks old broad bean plants using a brush, covered with a cellophane bag and maintained as described above for 48 hours. The adults were then removed and the nymphs (f_2 generation) maintained until they became adults, at which time they were inoculated with either *P. neoaphidis* or *B. bassiana* as described below.

Pandora neoaphidis

Inoculation arenas consisted of a Petri dish (9 cm i.d.) that was half filled with 1.5% tap-water–agar and had a broad bean leaf embedded abaxial side uppermost in the agar. Five dishes were prepared. Three wingless adult aphids (f_2 generation) from a single line were transferred to

each of the five Petri dish arenas. The aphids were then covered with Petri dishes (5 cm i.d.) containing the sporulating *P. neoaphidis* cadavers. These arenas were covered with a tissue paper followed by their lids (9 cm i.d.) and the three adult aphids were contaminated with *P. neoaphidis* conidia for 24 hours. During this time Petri dishes were enclosed in plastic bags containing wet filter paper to maintain a high relative humidity and promote infection. This was repeated for each of the fifteen lines simultaneously (seventy five inoculation chambers in total). As a control treatment, inoculation arenas were prepared as described above for each line but covered with a Petri dish (5 cm i.d.) containing only agar.

After 24 hours, all fifteen inoculated aphids (f_2 generation) from each line were transferred to new three-week-old broad bean plants (one plant per line, fifteen plants in total), covered with a cellophane bag and maintained at 18 ^{o}C, 16:8 L:D. Control aphids from the same line were also transferred to new bean plants (fifteen control plants in total). Aphids (f_2 generation) were observed daily for three days and then transferred to a Petri dish containing a bean leaf to ensure that they succumbed to fungal infection without infecting their offspring. The offspring (f_3 generation) were left on the plants until they became 4th instar nymphs or early adults at which point the morph type was determined.

Beauveria bassiana

Beauveria bassiana was cultivated on ten SDA plates under 23 ^{o}C. Conidia were scraped from the agar surface and mixed with 20 ml of 0.03% Tween 80 in a centrifuge tube. The suspension of conidia and mycelium was then vortexed for 5 minutes, filtered through muslin and the concentration of conidia determined using a Neubauer haemocytometer (6.1 x 10^8 conidia/ml). A bean leaf was then placed in a Petri dish (9 cm i.d.) and covered with 0.5 ml of the conidia suspension. Once dried, the leaf was transferred to another Petri dish which contained a filter paper (Whatman No 1) that had been soaked in a further 0.5 ml of the conidia suspension. As for *P. neoaphidis*, fifteen wingless aphids (f_2 generation) from each line were distributed equally among five Petri dishes containing the *B. bassiana*-treated leaf and filter paper and covered with a tissue paper and their lids. Petri dishes were enclosed in plastic bags for 24 hours with a wet filter paper and kept under controlled conditions (18^{o}C; 16:8 L:D). This procedure was repeated for all fifteen lines. As a control treatment, fifteen aphids (f_2 generation) from each line were maintained on five bean leaves treated with just 0.03% Tween 80 solution. After 24 hours, the aphids were transferred to bean plants as described previously. The adults were observed daily for three days and transferred to Petri dishes containing bean leaves and observed to ensure that they succumbed to fungal infection. The offspring were maintained and assessed as described previously. The *P. neoaphidis* and *B. bassiana* experiments were done simultaneously.

Experiment 2: Single aphids

Aphid lines

Seventeen aphid lines (f_0 generation) were established on bean leaves in Petri dishes as described above and were allowed to reproduce for 24 hours. Adults were then removed and their progeny (f_1 generation) maintained on leaves for ten days to become adults. The aphids (f_1 generation) were then inoculated with either *P. neoaphidis* or *B. bassiana* as described below.

Pandora neoaphidis

Prior to infection, *P. neoaphidis* was prepared to sporulate from infected aphid cadavers as described previously. One wingless adult aphid (f_1 generation) was transferred to an inoculation arena using a brush and covered with a Petri dish containing the sporulating cadavers. The infection procedure and abiotic conditions were the same as used for grouped aphids. This procedure was repeated for all seventeen lines. As a control treatment, seventeen aphids (f_1 generation; one per line) were transferred to Petri dish inoculation arenas and covered with a Petri dish containing only agar.

Aphids showered with *P. neoaphidis* conidia were individually transferred to new bean leaves in Petri dishes and covered with a tissue paper and their lids. Aphids were kept under controlled conditions (18^oC; 16:8 L:D) and observed daily. The adults were removed after three days to avoid infecting their offspring (f_2 generation) and transferred to new dishes and maintained individually to ensure that they succumbed to fungal infection. The offspring (f_2 generation) were left on leaves until they became 4^{th} instar nymphs or early adults at which point the number of nymphs was counted and the morph type determined.

Beauveria bassiana

Conidia from ten SDA plates were harvested, the conidia concentration determined (7.5 x 10^8 conidia/ml) and the broad bean leaves and filter paper soaked in the conidia suspension as described previously. As for *P. neoaphidis*, one wingless aphid (f_1 generation) from each of the seventeen lines was placed individually on a treated leaf and filter paper in a Petri dish and covered with a tissue paper followed by the lid. Petri dishes were enclosed in plastic bags with a wet filter paper to create a high relative humidity to allow infection and maintained under controlled conditions (18^oC; 16:8 L:D) for 24 hours. As a control, seventeen aphids (f_1 generation; one per line) were also kept individually on leaves and filter papers treated with only Tween 80 solution. Aphids treated with *B. bassiana* were transferred to new bean leaves in Petri dishes, maintained and their offspring (f_2 generation) assessed as described previously.

Statistical analysis

To analyse the effect of pathogen species (*P. neoaphidis* or *B. bassiana*), infection (infected or uninfected) on the proportion of winged morphs, a Generalized Linear Model (GLM) with a quasibinomial error distribution was used. Aphid lines were used as random effects while numbers of offspring were used as a covariate. Numbers of offspring produced were analysed by a GLM with a quasipoisson error distribution using aphid lines as random effects and pathogen species and infection as independent response variables. Models were simplified to the minimal adequate by removing non-significant interactions followed by independent variables if these were not included in any significant interaction (Crawley, 2007d). Among non-significant independent variables or interactions with the same number of variables, the one with the highest *p* value was removed first followed by the others in a descending order. After removing a parameter, the new model was only accepted if the removal did not significantly increase deviance compared to the previous model after a *F* test ($P>0.05$) (Crawley, 2007a, 2007b, 2007c). Otherwise, the previous model was retained and the simplification continued with the next non-significant interaction or variable. Data were analysed using R software 2.9.0 (www.r-project.org).

RESULTS

Experiment 1: Grouped aphids

Most treated adult aphids (f_2 generation) succumbed to infection by the end of this experiment: 12.80 $\pm$ 0.42 adults (85.33 $\pm$ 2.66%) succumbed to *P. neoaphidis* and 12.13 $\pm$ 0.61 adults (80.87 $\pm$ 4.07%) succumbed to *B. bassiana* on each plant. The remaining dead aphids did not sporulate.

The number of offspring produced during this experiment was not significantly affected by the pathogen species ($F_{1,57} = 1.475$, $P = 0.230$), infection ($F_{1,57} = 2.161$, $P = 0.147$), or the interaction between both factors ($F_{1,55} = 3.847$, $P = 0.055$; Fig. 1). There was also no significant difference among aphid lines ($F_{14,42} = 0.942$, $P = 0.525$).

Among aphids that were grouped, infected aphids produced a significantly higher proportion of winged offspring than uninfected aphids ($t_{57} = 2.116$, $P = 0.038$, Fig. 2B). There was a significant positive relationship between the total number of nymphs and the proportion that were winged ($F_{1,56} = 4.723$, $P = 0.03$, Fig. 2A). Neither pathogen species ($F_{1,67} = 1.527$, $P = 0.221$), aphid line ($F_{14,43} = 1.230$, $P = 0.292$) or any interaction between factors were significant.

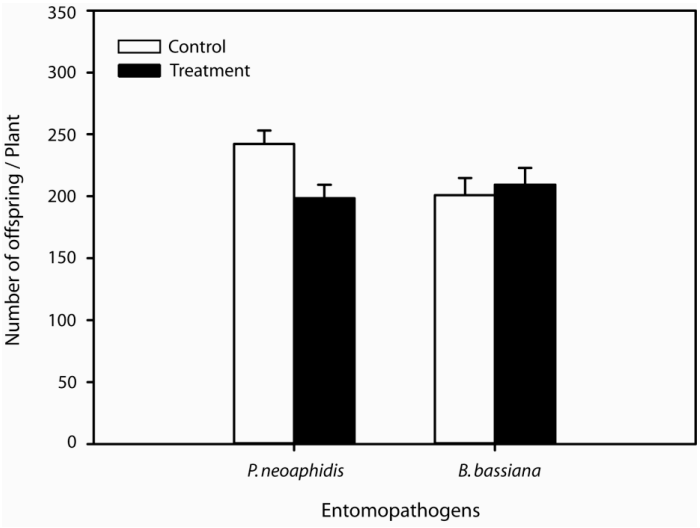

Figure 1. Number of offspring from groups of fifteen aphids inoculated with either *P. neoaphidis* or *B. bassiana* (black bars) or their respective controls (white bars). There was a significant effect of pathogen species on the number of offspring produced (t_{59} = 5.175, P < 0.001). The bars show mean values + SE.

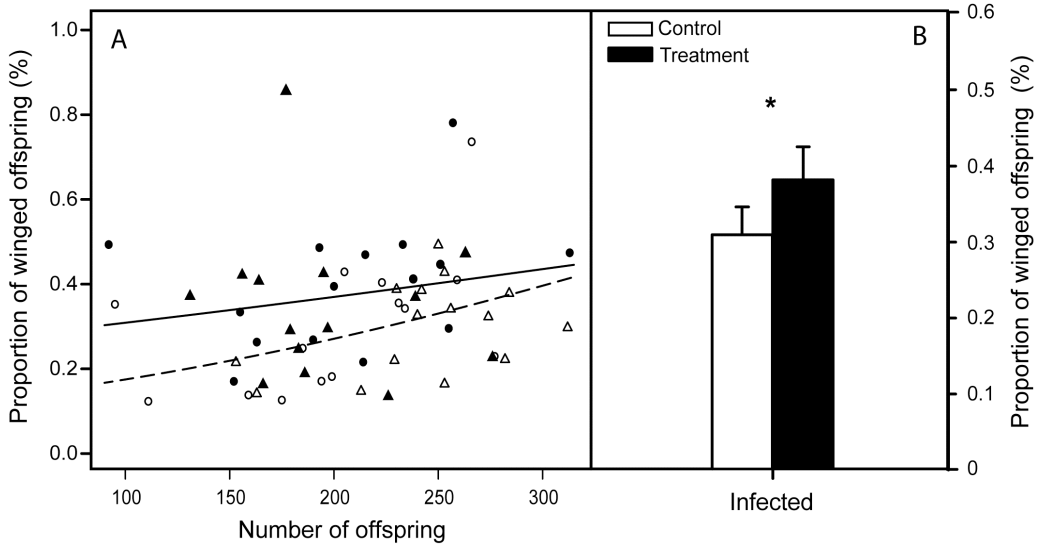

Figure 2. The relationship between the proportion of winged morphs and the number of offspring from groups of aphids treated with either *P. neoaphids* or *B. bassiana* or the respective controls (A). Open circles represent aphids treated with *P. neoaphidis* and open triangles represent its control; black triangles represent aphids treated with *B. bassiana* and black circles its control. Dashed line represents the linear regression over all control aphids and solid line represents all infected aphids. There is a significant effect of the number of offspring on the proportion of winged offspring independent of treatment (r^2 = 0.0065, $F_{1,56}$ = 4.723, P = 0.03). The proportion of winged offspring from grouped aphids infected with *P. neoaphids* or *B. bassiana* (black bar) and uninfected (white bar, P = 0.038) (B). The bars show mean values + SE.

Experiment 2: Single aphids

In this experiment all inoculated aphids (f_1 generation) succumbed to infection after being removed from leaves.

There was no significant difference between the number of offspring produced by infected or uninfected aphids ($F_{1,67}$ = 1.963, P = 0.054), or between pathogen species ($t_{1,66}$ = 1.981, P = 0.487). Aphid line ($F_{16,48}$ = 1.023, P = 0.453) and the interaction between pathogen species and infection ($F_{1,47}$ = 3.127, P = 0.084) were not significant either (Fig. 3).

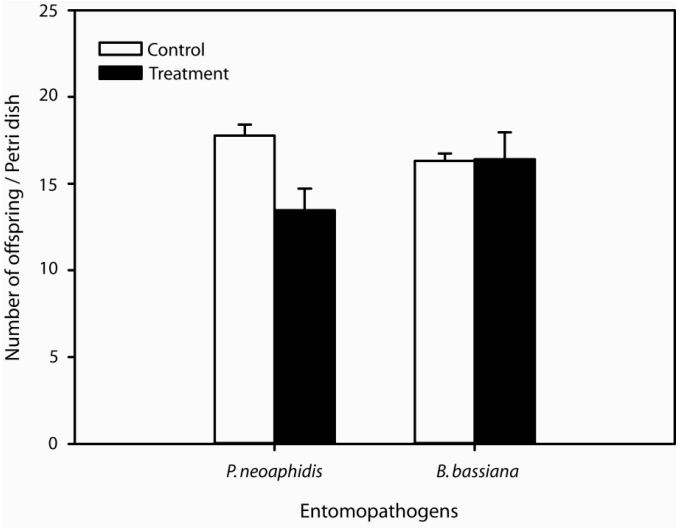

Figure 3. Number of offspring from isolated aphids treated with either *P. neoaphidis* or *B. bassiana* (black bars) or their respective controls (white bars). No variable or interaction was significant. The bars show mean values + SE.

The proportion of winged offspring was significantly affected by infection ($F_{1,65}$=10.301, P<0.01), in which infected aphids produced a higher proportion of winged offspring than uninfected aphids (Fig. 4B). Pathogen species, aphid line and number of offspring did not significantly affect wing induction ($F_{1,65}$=0.047, P=0.829; $F_{16,48}$=0.748, P=0.638 and $F_{1,64}$=0.843, P=0.859, respectively). Furthermore, none of the interactions were significant.

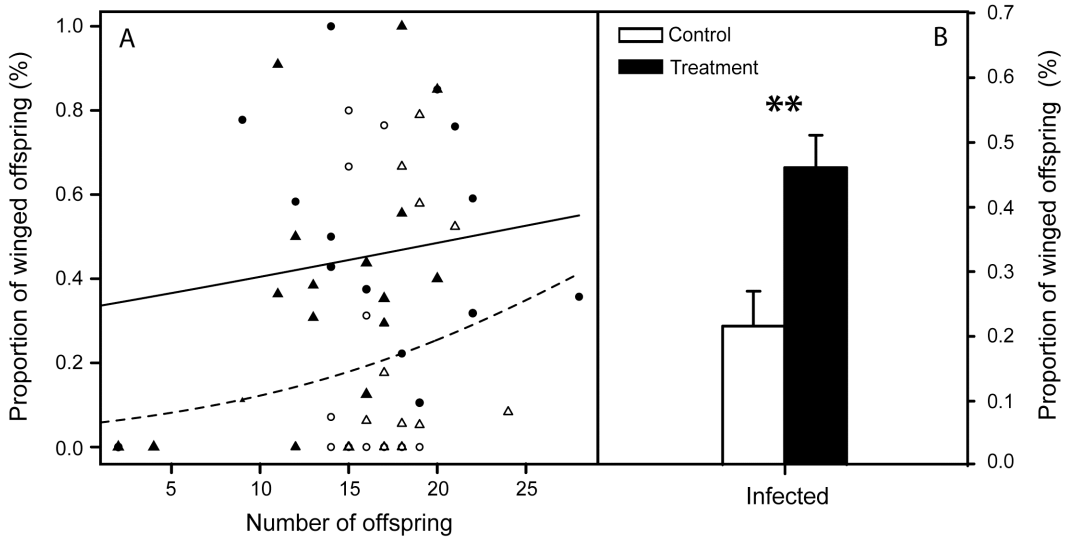

Figure 4. The relationship between the proportion of winged morphs and the number of offspring from groups of aphids treated with either *P. neoaphids* or *B. bassiana* or the respective controls (A). Open

circles represent aphids treated with *P. neoaphidis* and open triangles represent its control; black triangles represent aphids treated with *B. bassiana* and black circles its control. Dashed line represents the linear regression over all control aphids and solid line represents all infected aphids. There is no significant effect of the number of offspring on the proportion of winged offspring independent of treatment (r^2 = 0.008, F = 0.843, P = 0.365). The proportion of winged offspring from grouped aphids infected with *P. neoaphids* or *B. bassiana* (black bar) and uninfected (white bar) (B). The bars show mean values + SE.

DISCUSSION

Pea aphids are predominantly wingless insects that produce winged offspring under adverse conditions such as predation risk, high population density or low nutrition of host plants. We have demonstrated that the entomopathogenic fungi *P. neoaphidis* and *B. bassiana* affected adult aphids resulting in the induction of wing formation in their offspring. Unlike wing induction triggered by predators and parasitoids, the effect of entomopathogenic fungi did not require physical contact with conspecifics and, consequently, is not mediated by the pseudo crowding effect. Interestingly, wing induction was not different between pathogen species suggesting the same mechanism triggers wing formation.

Wings facilitate dispersal to explore new patches and to propagate the genotype whilst minimizing competition and predation levels. Although there is a potential fitness cost of inducing wings through a lower reproduction rate and longer development time (Dixon, 1998b), this morphological modification is functionally important for aphids to maximise reproductive success by leaving patches containing entomopathogenic fungi. This phenomenon may also indirectly benefit the infected mother: by enhancing reproduction success of the progeny, mothers will increase gene frequency of the clone in a population (Alcock, 1998; Hamilton, 1964; West-Eberhard, 1989).

However, it is also possible that pathogens also benefit from the wing induction: aphids can move to a new host to avoid infection sites, but they can also vector conidia, facilitating pathogen dispersal to new patches and contaminating a new or an existing colony, as has been observed for other species (Meyling *et al.*, 2006; Roy *et al.*, 2001; Roy *et al.*, 1998). Furthermore, winged morphs of *Myzus persicae* (Sulzer), *Brevicoryne brassicae* (L.) and *Lypaphis erysimi* (Kaltenbach) captured from the field were already infected by entomopathogenic fungi including *P. neoaphidis*, which subsequently established in young aphid colonies (Feng *et al.*, 2007; Huang *et al.*, 2008). Although there is the risk that pathogens may not find a host colony immediately, conidia can remain viable in the soil or on the plant surface until they encounter a host (Baverstock *et al.*, 2008b). Furthermore, the chance of finding a suitable host in a new patch may be increased through targeted vectoring by insects, such as the aphid tending ant, *Lasius niger* L. (Bird *et al.*, 2004), the predatory ladybird, *C. septempunctata* (Pell *et al.*, 1997; Roy *et al.*, 2001), and the predatory bug, *Orius laevigatus* (Fieber) (Down *et al.*, 2009). In addition, *B. bassiana* can also use insects from other orders as hosts.

Roy *et al.* (2005) showed that pea aphids infected with *P. neoaphidis* and *B. bassiana* emit alarm pheromone, but at a different dynamic to each other: *P. neoaphidis*-infected aphids

increase the amount of alarm pheromone emitted from infection until conidiation, whilst *B. bassiana*-infected aphids do the opposite. This fact could raise the hypothesis that the wing induction observed here was indirectly mediated by alarm pheromone in our grouped aphid experiment via the pseudo crowding effect, as when aphids are attacked by arthropod natural enemies. However, the direct effect of entomopathogenic fungi on aphids is still determinant for wing induction since aphids that were infected and maintained in isolation also produced winged offspring. This is in accordance with the work of Kunert *et al.* (2005) who demonstrated that isolated control aphids and pea aphids exposed to alarm pheromone did not differ in wing induction. Therefore, the effect of alarm pheromone alone could not have triggered the wing induction in infected pea aphids. Although it is possible that the pseudo crowding effect might be in part involved in wing induction in our experiment with grouped aphids, it is still unlikely that a constant release of alarm pheromone had a strong effect on the aphid behaviour. Once feeding, infected aphids do not show the typical alarm behaviour of removing the stylet, walking or dropping from plants (personal observation) that leads to the pseudo crowding effect. It is likely that aphids do not react because they become habituated to a constant release of (*E*)-β-farnesene (Petrescu *et al.*, 2001), the component of the pea aphid alarm pheromone, as it is also released from plants (Bruce *et al.*, 2005; Dawson *et al.*, 1984; Gibson & Pickett, 1983) and respond only to rapid pulses emitted when an aphid is attacked and releases droplets.

Crowding was, however, significant for wing induction and worked independently with infection in our group experiment. The role of crowding in aphid wing induction was described not only for *A. pisum* (Sutherland, 1969a), but for many other aphid species, including *Megoura viciae* Buckton (Lees, 1967), *M. persicae* (Sutherland & Mittler, 1971), and *A. fabae* (Shaw, 1970a). Because infection was not significant for wing induction, we can assume that infected and uninfected aphids were equally sensitive to physical contact.

Since *P. neoaphidis* and *B. bassiana* triggered wing formation in our single aphid experiment, entomopathogenic fungi do not induce wing formation via pseudo crowding as do arthropod natural enemies. Thus, fungal infection is a cue to induce wing formation in *A. pisum* and contrasts with arthropod parasitism which does not directly promote wing induction (Sloggett & Weisser, 2002). In the case of parasitoid attack, it was found that pea aphids do not physiologically respond to eggs of the parasitoid *A. ervi* deposited in their bodies by producing winged offspring (Sloggett & Weisser, 2002). However, direct physiological manipulation for wing induction by a parasite was observed when the rosy apple aphid, *Disaphis plantaginea* (Passerini), was infected with the *Disaphis plantaginea* densovirus (DpIDNV) (Ryabov *et al.*, 2009). This virus was essential to induce wings since virus-free aphids did not produce any winged offspring, even in crowding conditions or poor plant quality (Ryabov *et al.*, 2009). As this virus increases the proportion of winged offspring and mobility of aphids, it is likely that it also benefits viral transmission (Ryabov *et al.*, 2009). Therefore, there are potentially three mechanisms for wing induction triggered by entomopathogenic fungi. First, the pathogens have an active role in wing induction by directly introducing metabolites which affect wing induction in embryos or the mother's physiology. Second, wing induction is a response of aphid mothers to pathogen infection as an immunological response when the parasite invades the body. Finally, it is possible that the pathogens' conidia in contact with aphids would mechanically stimulate wing

induction. In pseudo crowding and crowding conditions, the hairs and bristles on the head, antenna, legs and body are important channels to detect physical stimuli and induce wing formation in offspring (Johnson, 1965; Kunert & Weisser, 2005; Lees, 1967; Sutherland, 1969a). Thus it is possible that during infection or germination, conidia contacted these hairs and mechanically stimulated the aphid to trigger the wing formation.

In conclusion, we present evidence that the entomopathogenic fungi *P. neoaphidis* and *B. bassiana* induce wing formation in aphids and that this phenomenon is independent of the pseudo crowding effect that occurs when a colony is attacked by predators and parasitoids. Although *P. neoaphidis* is a specialist pathogen of aphids and is under different selection pressures to the generalist pathogen *B. bassiana*, they both trigger wing induction equally in *A. pisum*, suggesting that the mechanism is the same. Whilst it is advantageous for aphids to leave infected patches it is also likely that entomopathogenic fungi benefit by using winged aphids as vectors for dispersal. The ecological costs and biochemical pathways for these fungi to trigger wing induction remain to be elucidated.

ACKNOWLEDGEMENTS

We wish to thank Patricia Wells and Helen E. Roy for kindly helping to infect aphids with pathogens. This study was supported by the International Max Planck Research School in Jena and the Department for Environment, Food and Rural Affairs of the United Kingdom (Defra). Rothamsted Research is an Institute of the Biotechnology and Biological Sciences Research Council of the United Kingdom.

REFERENCES

Alcock, J. (1998). The adaptive value of social living. In Animal behavior: an evolutionary approach (ed J. Alcock), pp. 555-602. Sinauer Associates, Inc., Sunderland, Massachusetts.

Ankersmit, G.W. & Dijkman, H. (1983) Alatae production in the cereal aphid *Sitobion avenae*. Neth. J. Plant Pathol. , 89, 105-112.

Baverstock, J., Alderson, P.G., & Pell, J.K. (2005) Influence of the aphid pathogen *Pandora neoaphidis* on the foraging behaviour of the aphid parasitoid *Aphidius ervi*. Ecol. Entomol., 30, 665-672.

Baverstock, J., Baverstock, K.E., Clark, S.J., & Pell, J.K. (2008a) Transmission of *Pandora neoaphidis* in the presence of co-occurring arthropods. J. Invertebr. Pathol., 98, 356-359.

Baverstock, J., Clark, S.J., Alderson, P.G., & Pell, J.K. (2009) Intraguild interactions between the entomopathogenic fungus *Pandora neoaphidis* and an aphid predator and parasitoid at the population scale. J. Invertebr. Pathol., 102, 167-72.

Baverstock, J., Clark, S.J., & Pell, J.K. (2008b) Effect of seasonal abiotic conditions and field margin habitat on the activity of *Pandora neoaphidis* inoculum on soil. J. Invertebr. Pathol., 97, 282-290.

Bird, A.E., Hesketh, H., Cross, J.V., & Copland, M. (2004) The common black ant, *Lasius niger* (Hymenoptera : Formicidae), as a vector of the entomopathogen *Lecanicillium longisporum* to rosy apple aphid, *Dysaphis plantaginea* (Homoptera : Aphididae). Biocontrol Sci. Technol., 14, 757-767.

Bruce, T.J.A., Birkett, M.A., Blande, J., Hooper, A.M., Martin, J.L., Khambay, B., Prosser, I., Smart, L.E., & Wadhams, L.J. (2005) Response of economically important aphids to components of *Hemizygia petiolata* essential oil. Pest Manag. Sci., 61, 1115-1121.

Crawley, M.J. (2007a). Count data. In The R book (ed M.J. Crawley), pp. 527-548. John Wiley & Sons Ltd, Chichester.

Crawley, M.J. (2007b). Generalized linear models. In The R book (ed M.J. Crawley), pp. 511-526. John Wiley & Sons Ltd, Chichester.

Crawley, M.J. (2007c). Proportion data. In The R book (ed M.J. Crawley), pp. 569-626. John Wiley & Sons Ltd, Chichester.

Crawley, M.J. (2007d). Statistical modelling. In The R book (ed M.J. Crawley), pp. 323-386. John Wiley & Sons Ltd, Chichester.

Dawson, G.W., Griffiths, D.C., Pickett, J.A., Smith, M.C., & Woodcock, C.M. (1984) Natural Inhibition of the aphid alarm pheromone. Entomol. Exp. Appl., 36, 197-199.

Dixon, A.F.G. (1998a). Resource tracking in time. In Aphid ecology (ed A.F.G. Dixon), pp. 128-170. Chapman & Hall, London.

Dixon, A.F.G. (1998b). Resource tracking: mechanism - polyphenism. In Aphid ecology (ed A.F.G. Dixon), pp. 100-127. Chapman & Hall, London.

Dixon, A.F.G. & Agarwala, B.K. (1999) Ladybird-induced life-history changes in aphids. Proc. R. Soc. Lond. Ser. B-Biol. Sci., 266, 1549-1553.

Down, R.E., Cuthbertson, A.G.S., Mathers, J.J., & Walters, K.F.A. (2009) Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus*, to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci*. Biol. Control, 50, 172-178.

Feng, M.G., Chen, C., Shang, S.W., Ying, S.H., Shen, Z.C., & Chen, X.X. (2007) Aphid dispersal flight disseminates fungal pathogens and parasitoids as natural control agents of aphids. Ecol. Entomol., 32, 97-104.

Gibson, R.W. & Pickett, J.A. (1983) Wild potato repels aphids by release of aphid alarm pheromone. Nature, 302, 608-609.

Hamilton, W.D. (1964) Genetical evolution of social behaviour 2. J. Theor. Biol., 7, 17-52.

Hemmati, F., Pell, J.K., McCartney, H.A., & Deadman, M.L. (2001) Airborne concentrations of conidia of *Erynia neoaphidis* above cereal fields. Mycol. Res., 105, 485-489.

Hodgson, E.W., Venette, R.C., Abrahamson, M., & Ragsdale, D.W. (2005) Alate production of soybean aphid (Homoptera : Aphididae) in Minnesota. Environ. Entomol., 34, 1456-1463.

Huang, Z.H., Feng, M.G., Chen, X.X., & Liu, S.S. (2008) Pathogenic fungi and parasitoids of aphids present in air captures of migratory alates in the low-latitude plateau of Yunnan, China. Environ. Entomol., 37, 1264-1271.

Johnson, B. (1965) Wing polymorphism in aphids II. Interaction between aphids. Entomol. Exp. Appl., 8, 49-64.

Johnson, B. (1966) Wing polymorphism in aphids III. Influence of host plant. Entomol. Exp. Appl., 9, 213-222.

Kunert, G., Otto, S., Rose, U.S.R., Gershenzon, J., & Weisser, W.W. (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecol. Lett., 8, 596-603.

Kunert, G., Schmoock-Ortlepp, K., Reissmann, U., Creutzburg, S., & Weisser, W.W. (2008) The influence of natural enemies on wing induction in *Aphis fabae* and *Megoura viciae* (Hemiptera: Aphididae). B. Entomol. Res., 98, 57-62.

Kunert, G. & Weisser, W.W. (2003) The interplay between density-and trait-mediated effects in predator-prey interactions: a case study in aphid wing polymorphism. Oecologia, 135, 304-312.

Kunert, G. & Weisser, W.W. (2005) The importance of antennae for pea aphid wing induction in the presence of natural enemies. B. Entomol. Res., 95, 125-131.

Lees, A.D. (1967) Production of apterous and alate forms in aphid *Megoura viciae* Buckton with special reference to role of crowding. J. Insect Physiol., 13, 289-318.

Meyling, N.V., Pell, J.K., & Eilenberg, J. (2006) Dispersal of *Beauveria bassiana* by the activity of nettle insects. J. Invertebr. Pathol., 93, 121-126.

Minks, A.K. & Harrewijn, P. (1987). Biology. In Aphids, their biology, natural enemies and control (eds A.K. Minks & P. Harrewijn), Vol. 2A, pp. 197-297. Elsevier, Amsterdam.

Mondor, E.B., Rosenheim, J.A., & Addicott, J.F. (2005) Predator-induced transgenerational phenotypic plasticity in the cotton aphid. Oecologia, 142, 104-108.

Pell, J.K., Eilenberg, J., Hajek, A.E., & Steinkraus, D.S. (2001). Biology, ecology and pest management potential of entomophthorales. In Fungi as Biocontrol Agents: Progress, Problems and Potential (eds T.M. Butt, C. Jackson & N. Magan), pp. 71-154. CABI International.

Pell, J.K., Pluke, R., Clark, S.J., Kenward, M.G., & Alderson, P.G. (1997) Interactions between two aphid natural enemies, the entomopathogenic fungus *Erynia neoaphidis* Remaudiere & Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L (Coleoptera: Coccinellidae). J. Invertebr. Pathol., 69, 261-268.

Petrescu, A.S., Mondor, E.B., & Roitberg, B.D. (2001) Subversion of alarm communication: Do plants habituate aphids to their own alarm signals? Can. J. Zool., 79, 737-740.

Podjasek, J.O., Bosnjak, L.M., Brooker, D.J., & Mondor, E.B. (2005) Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*. Can. J. Zool., 83, 1138-1141.

Roy, H.E. & Cottrell, T.E. (2008) Forgotten natural enemies: Interactions between coccinetlids and insect-parasitic fungi. Eur. J. Entomol., 105, 391-398.

Roy, H.E. & Pell, J.K. (2000) Interactions between entomopathogenic fungi and other natural enemies: Implications for biological control. Biocontrol Sci. Technol., 10, 737-752.

Roy, H.E., Pell, J.K., & Alderson, P.G. (2001) Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. Biocontrol Sci. Technol., 11, 99-110.

Roy, H.E., Pell, J.K., Clark, S.J., & Alderson, P.G. (1998) Implications of predator foraging on aphid pathogen dynamics. J. Invertebr. Pathol., 71, 236-247.

Ryabov, E.V., Keane, G., Naish, N., Evered, C., & Winstanley, D. (2009) Densovirus induces winged morphs in asexual clones of the rosy apple aphid, *Dysaphis plantaginea*. Proc. Natl. Acad. Sci. U. S. A., 106, 8465-8470.

Schwartzberg, E.G., Kunert, G., Roese, U.S.R., Gershenzon, J., & Weisser, W.W. (2008) Alarm pheromone emission by pea aphid, *Acyrthosiphon pisum*, clones under predation by lacewing larvae. Entomol. Exp. Appl., 128, 403-409.

Shaw, M.J.P. (1970a) Effects of population density on alienicolae of *Aphis fabae* Scop .1. Effect of crowding on production of alatae in laboratory. Ann. Appl. Biol., 65, 191-196.

Shaw, M.J.P. (1970b) Effects of population density on alienicolae of *Aphis fabae* Scop .3. Effect of isolation on development of form and behaviour of alatae in a laboratory clone. Ann. Appl. Biol., 65, 205-212.

Shimazu, M., Sato, H., & Maehara, N. (2002) Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (Deuteromycotina : Hyphomycetes) in forest air and soil. Appl. Entomol. Zoolog., 37, 19-26.

Sloggett, J.J. & Weisser, W.W. (2002) Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*. Oikos, 98, 323-333.

Sloggett, J.J. & Weisser, W.W. (2004) A general mechanism for predator- and parasitoid-induced dispersal in the pea aphid, *Acyrthosiphon pisum* (Harris). In 6th International Symposium on Aphids (eds J.-C. Simon, C.A. Dedryver, C. Rispe & M. Hullé), pp. 79-85, Institut National de la Recherche Agronomique, Rennes, France.

Sutherland, O.R.W. (1967) Role of host plant in production of winged forms by a green strain of pea aphid *Acyrthosiphon pisum* Harris. Nature, 216, 387-388.

Sutherland, O.R.W. (1969a) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol., 15, 1385-1410.

Sutherland, O.R.W. (1969b) The role of host plant in production of winged forms by two strains of pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol., 15, 2179-2201.

Sutherland, O.R.W. & Mittler, T.E. (1971) Influence of diet composition and crowding on wing production by aphid *Myzus persicae*. J. Insect Physiol., 17, 321-328.

Völkl, W., Mackauer, M., Pell, J.K., & Brodeur, J. (2007). Predators, parasitoids and pathogens. In Aphid as crop pests (eds H.F.v. Emden & R. Harrington), pp. 187-234. CAB International, Wallingford.

Weisser, W.W. & Braendle, C. (2001) Body colour and genetic variation in winged morph production in the pea aphid. Entomol. Exp. Appl., 99, 217-223.

Weisser, W.W., Braendle, C., & Minoretti, N. (1999) Predator-induced morphological shift in the pea aphid. P. Roy. Soc. Lond. B Bio., 266, 1175-1181.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst., 20, 249-278.

Wilding, N. (1970) The effect of temperature on the infectivity and incubation periods of the fungi *Entomophthora aphidis* and *Entomophthora thaxteriana* for the pea aphid *Acyrthosiphon pisum*. In International Colloquium on Insect Pathology Proceedings, Vol. 4, pp. 84-88.

Wilding, N. & Perry, J.N. (1980) Studies on *Entomophthora* in populations of *Aphis fabae* on field beans. Ann. Appl. Biol., 94, 367-378.

Williams, I.S. & Dixon, A.F.G. (2007). Life cycles and polymorphism. In Aphids as crop pests (eds H.F.v. emden & R. Harrington), pp. 69-86. CAB International, Wallingford.

3. GENERAL DISCUSSION

Since the 1970's the aphid alarm pheromone (*E*)-β-farnesene has been studied in order to understand the ecology of aphids, first as a repellent to many aphids species, followed by its application in crop protection, and finally because of its attractiveness to aphid predators and parasitoids. Until ten years ago, the ecology behind the phenotypic plasticity of aphids to produce winged morphs was restricted to the effects of plant nutritional quality, crowding and environmental conditions, and never crossed the alarm behaviour. Since then, (*E*)-β-farnesene has been gaining more attention in its new role as a mediator of wing induction by natural enemies via the pseudo crowding effect. The experiments in this thesis contribute to our further understanding of the ecological role of this alarm signal in interactions between aphids and their natural enemies and show how the role becomes more complex when different organisms are included. While (*E*)-β-farnesene may benefit the survival of aphid colonies by making them disperse or producing winged morphs, it also bears important fitness costs for aphids when alarmed and by attracting natural enemies.

While the mechanism by which the alarm pheromone mediates wing induction in pea aphids was clearly described in Kunert *et al.* 2005 and 2007, aphids may respond differently under laboratory and field conditions. This mechanism was then tested under natural environmental conditions in a field experiment and the phenomenon was reproduced, confirming that the aphid alarm pheromone is the active signal that mediates wing induction when pea aphids are disturbed by their natural enemies (Manuscript I).

Kunert *et al.* (2007) found that the perception of (*E*)-β-farnesene is not dependent on the population density to stimulate the production of winged offspring. Although Hatano *et al.* (Manuscript I) found the same for the interaction of these two variables, when the effect of location (field or climate chamber) is considered, the interaction among these three variables becomes significant. This suggests that the emission of (*E*)-β-farnesene may have an effect on wing induction according to population density and depending on environmental conditions. This work, therefore, reinforces the argument that ecological phenomena may proceed differently under natural environmental conditions.

Although the applied amount of (*E*)-β-farnesene (1 µg) was higher than values found by Schwartzberg *et al.* (2008) and Mondor *et al.* (2000), it is not likely that these results were overestimated. As presented by Kunert *et al.* (2005), the frequency with which (*E*)-β-farnesene was applied, which reflects the frequencies of predation events, was more important for wing induction than the amount of (*E*)-β-farnesene applied. The reason for applying more than natural amounts of (*E*)-β-farnesene in the field test was to level out the different distances between the (*E*)-β-farnesene source and receivers. In a colony, attacked aphids alarm nearby conspecifics and, therefore, the concentration of (*E*)-β-farnesene that reaches the aphids' antennae is high. In

the field experiment, (*E*)-β-farnesene was applied to filter paper close to the soil and, therefore, the distance to the aphids was much longer. Furthermore, wind, temperature and humidity probably had a smaller effect on aphid-aphid communication because of the short distance between aphids compared to the longer distance between (*E*)-β-farnesene source and aphid receivers.

While (*E*)-β-farnesene benefits an aphid colony by inducing members to escape from a habitat with a high risk of predation, under natural conditions it also carries ecological costs for aphids: the risk of dispersal and the risk of attracting natural enemies. In the field experiment, aphid colonies exposed to (*E*)-β-farnesene decreased in size faster than undisturbed colonies (Manuscript I). Therefore, it is likely that (*E*)-β-farnesene might also increase mortality by starvation or desiccation when aphids abandon their feeding site (Dill *et al.*, 1990; Losey & Denno, 1998; Roitberg & Myers, 1978). These costs of responding to alarm pheromones may vary according to aphid species (Dixon, 1958), their age (Losey & Denno, 1998), clone type (Braendle & Weisser, 2001; Müller, 1983), host plant (Stadler *et al.*, 1994), aphid host race (Kunert *et al.*, 2010), mutualism with ants (Xiangyu *et al.*, 2002) and predator species (Losey & Denno, 1998) because these factors influence the propensity of aphids to drop.

Another cost for the survival of aphid colonies when emitting alarm pheromone is the attraction of natural enemies that perceive this cue to locate aphids. It is natural that predators and parasitoids use (*E*)-β-farnesene since it is a reliable cue widely shared among many aphid species (Francis *et al.*, 2005). After locating a patch by detecting the volatiles from the host plant, generalist and specialist natural enemies use (*E*)-β-farnesene to locate aphids on a plant (Manuscript II). A difficulty that natural enemies face is to find (*E*)-β-farnesene at detectable levels, since this signal is emitted in low amounts by aphids. This is, however, advantageous for aphids to try to remain chemically inconspicuous to natural enemies.

In a food web where organisms of different trophic levels interact, natural enemies may not only inter- and intraspecifically compete among themselves for the same resource, but they may also predate on each other (intraguild predation) (Polis *et al.*, 1989). This is especially the case for generalist predators that feed on a range of insect species from various trophic levels. The increase in number and diversity of interactions may open new opportunities for other organisms to adaptively explore the (*E*)-β-farnesene from aphids for their benefit. In addition to the kairomone role of (*E*)-β-farnesene for natural enemies, a new role for (*E*)-β-farnesene was hypothesised when the behavioural response of the predatory gallmidge *A. aphidimyza* to the aphid alarm pheromone was studied (Manuscript III). *A. aphidimyza* larvae do not cause aphids to emit cornicle droplets because of their furtive feeding strategy. However, they are attacked by intraguild predators of aphids because larvae have soft bodies and move slowly. When exposed to (*E*)-β-farnesene, this predator started dispersing independently of aphid alarm behaviour. Therefore, for *A. aphidimyza,* (*E*)-β-farnesene is not a sign of the presence of intraspecific competitors in the colony but indicates a risk of predation as it does for aphids. Since the (*E*)-β-farnesene emitted by aphids also benefits the receiver *A. aphidimyza*, (*E*)-β-farnesene can be

classified as a synomone, that is, a chemical that is emitted by an organism and perceived by another of a different species and evokes a behavioural or physiological reaction adaptively favourable to both organisms (Nordlund & Lewis, 1976). The perception of (*E*)-β-farnesene is probably an innate rather than a learned trait of larvae because the cost would be high if the larvae failed to relate (*E*)-β-farnesene to the presence of predators. In fact, *A. aphidimyza* is a specialist predator (Nijveldt, 1988) and, therefore, may have closely evolved with aphids and their omnivorous predators to use (*E*)-β-farnesene for its own benefit.

Because the effect of (*E*)-β-farnesene varies with population density under natural conditions to promote aerial dispersal (Manuscript I), and the larger the colony is, the higher is the risk of being chemically and physically conspicuous to natural enemies (Manuscript II), it is reasonable to think that alarm signalling is not used indiscriminately but has evolved to be regulated by the signaller according to ecological conditions. Such regulation would balance costs and benefits to favour aphid colonies. The ecology of alarm communication shows that, in order to minimize the costs, alarm calls are regulated according to group size, the predator and the degree of kin relation (Alcock, 1998b). Belding's group squirrels, for instance, produce different alarm calls when they spot an aerial or ground predator, and the calls vary according to the squirrels' sex, category of reproduction stage and presence of kin (Alcock, 1998b). Social conditions may also affect morphological traits for defence as it is observed in size of shrimps' claws (Tóth & Duffy, 2008), production of spines in marine bryozoans (Harvell, 1992), production of soldiers in gall aphids (Shibao, 1997), and aggressive responses as for wasps (London & Jeanne, 2003) and honeybees (Schneider & McNally, 1992). It is thus likely that similar social evolutionary pressures produced changes in induced defences of organisms.

In order to investigate whether pea aphids regulate their alarm call according to their social conditions, four mechanisms were hypothesized:

I) Aphids emit (*E*)-β-farnesene in response to perceiving (*E*)-β-farnesene: when neighbouring aphids perceive the (*E*)-β-farnesene from an attacked aphid, they also emit alarm pheromone to amplify the alarm signalling. This signal cascade would then warn further aphids in a colony of the presence of a natural enemy on a plant.

II) The amount of (*E*)-β-farnesene emitted is dependent of the amounts stored in the body: when perceiving cues that reflect the social conditions of a colony, aphids would regulate the production of (*E*)-β-farnesene according to group size. The amounts of (*E*)-β-farnesene stored would then affect the emission of this pheromone.

III) The emission of (*E*)-β-farnesene is determined by the size of the colony: in this case, the production of (*E*)-β-farnesene is not affected by social conditions and aphids regulate the amounts emitted when exposing the cornicle droplet.

IV) The emitted and/or stored amounts of (*E*)-β-farnesene are dependent of the weight of the aphid: social conditions would affect the aphid growth rate which affects the aphid fitness to produce and/or emit (*E*)-β-farnesene.

By using a qualitative approach, a deuterated (*E*)-β-farnesene, which can be analytically differentiated from (*E*)-β-farnesene, was applied to aphid colonies. If hypothesis I were true, pea aphids would emit natural (*E*)-β-farnesene when perceiving the deuterated (*E*)-β-farnesene and the GC-MS analysis of collected volatiles would detect two peaks with a few seconds' difference in their retention time and different *m/z* values. Although the deuterated (*E*)-β-farnesene triggered the same alarm behaviour and the production of winged offspring of natural (*E*)-β-farnesene, undisturbed aphids do not emit further (*E*)-β-farnesene, suggesting that they do not amplify the signal like a signal cascade response (Manuscript IV).

Further experiments to study the alarm signal given by the attacked aphid demonstrated that (*E*)-β-farnesene emission was regulated according to different social conditions: isolated pea aphids released higher amounts of (*E*)-β-farnesene than did grouped ones. However, this emission of (*E*)-β-farnesene was not correlated to the amount stored within the aphid body. In fact, analysis of body extracts found no difference in the amounts of stored (*E*)-β-farnesene between aphids under two different social conditions, falsifying our second hypothesis (Manuscript V). Although social conditions affected the weight of aphids and this affected the stored amounts of alarm pheromone (with smaller aphids producing more (*E*)-β-farnesene than heavier ones), the amounts of (*E*)-β-farnesene emitted was not regulated by the aphid size, falsifying hypothesis IV. Therefore, the emission of alarm pheromone is regulated according to social conditions around the aphid when emitting the cornicle droplet (hypothesis III). It is likely that large aphid colonies benefit from lowering the amount of alarm pheromone emitted to reduce the risk of attracting natural enemies. In contrast, small aphid colonies have lower chances of surviving an attack than larger colonies (West-Eberhard, 1989) and the benefits of emitting large amount of alarm pheromone would outweight the risk of attracting other natural enemies.

Interestingly, Verheggen *et al.* (2009) found that the regulatory system of pea aphids was similar to that of Belding's group squirrels when emitting (*E*)-β-farnesene; this response is enhanced in the presence of a large conspecific group, decreasing when in a small colony. The following points may have caused the differences between these two investigations:

I) In the collection method used by Verheggen *et al.* (2009), pea aphids were smashed in the volatile collection chamber, while in Hatano *et al.* (Manuscript VI), aphids were subjected to third instar larvae of the lacewing *C. carnea*. It is likely that aphids differ in their alarm communication according to stimuli-like attack and the feeding strategies of natural enemies (Frechette *et al.*, 2008; Losey & Denno, 1998; Lucas & Brodeur, 2001; Schwartzberg *et al.*, 2008b).

II) The volatiles were collected from aphids at different physiological adult stages in both experiments and this influenced the amount of (*E*)-β-farnesene emitted. While in Verheggen *et al.* (2009), tested pea aphids were pre-reproductive adults, in Hatano *et al.* (Manuscript V) the alarm pheromone from reproductive adults which emitted very similar amounts of (*E*)-β-farnesene were found in cornicle droplets from reproductive pea aphids (Mondor *et al.*, 2000).

Although this hypothesis still needs to be tested for different adult stages, Mondor *et al.* (2000) and Schwartzberg *et al.* (2008) have provided evidence that the amount of (*E*)-β-farnesene emitted by pea aphids is age dependent and declines from nymphs to adults; adults had the lowest amount.

Nevertheless, pea aphids can regulate the emission of alarm pheromone according to the presence of conspecifics and there is the possibility that this regulation, like the production of (*E*)-β-farnesene, changes according to aphid age and predator species. Furthermore, the amounts of alarm pheromone emitted in each social condition were demonstrated to induce wing formation according to the presence of conspecifics even at low population densities (Manuscript V). This suggests that the regulation of alarm pheromone emission is adaptive for aphids to reduce the risk of predation while still allowing the dispersal of conspecifics and production of winged morphs through pseudo crowding.

While this mechanism for wing induction mediated by (*E*)-β-farnesene is true for natural enemies that cause aphids to emit cornicle droplets, we lack knowledge about other natural enemies that do not cause the alarm behaviour. The term 'aphid natural enemies' usually refers to arthropod predators and parasitoids, but another class of natural enemy is often forgotten when studying food webs: the pathogens (Roy & Cottrell, 2008). Like arthropod natural enemies, these may be divided into generalists and specialists.

Since the mechanism that triggers wing formation by arthropods is closely dependent on physical contact among alarmed aphids, initially pathogens were hypothesised to have a null or negative effect on wing induction for two reasons: first, because aphids infected by the pathogens' conidia or nearby aphids do not show any alarm behaviour; and second, because some pathogens reduce the sensitivity of aphids to alarm pheromones, reducing their dispersal behaviour (Roy *et al.*, 2005; Roy *et al.*, 1999). When assessing the effect of an aphid specific pathogen, *Pandora neoaphidis*, and a generalist insect pathogen, *Beauveria bassiana*, it was observed that aphids infected with these pathogenic fungi actually induced wing formation (Manuscript VI). Unlike the arthropods' mechanism, the pathogenic mechanism that induces wings is not linked to pseudo crowding, because single aphids also produce a high proportion of winged offspring. Pathogens, therefore, directly induce wing formation in pea aphids.

The wing induction may have two functional consequences for pathogens as well as aphids. First, wing induction could benefit the pathogens by using winged morphs as vectors (Feng *et al.*, 2007; Huang *et al.*, 2008). Although the chances of finding a new host colony by dispersing with winged morphs is low, vectoring aphids would still land on a suitable host plant and conidia would likely use a sit-and-wait strategy until a host aphid arrives and gets infected. Second, wing induction is a response from infected pea aphids to disperse from infected patches, which increase the reproductive success. This hypothesis is more likely since the fungi have evolved to produce hundreds of thousands of conidia that can be dispersed by wind. Further studies to observe whether winged offspring obtain conidia and what their fate is will help to better

understand the ecological role of wing induction for pathogens.

We can now answer the questions in the introduction:

1. The aphid alarm pheromone mediates the maternally induced wing formation in offspring under field conditions. This phenotypic change may enhance the chances of successful reproduction of the clone. The alarm behaviour that helps aphids to avoid encountering a natural enemy may also increase mortality rate while foraging for a new host plant.
2. (*E*)-β-farnesene plays an important kairomone role for many natural enemies. It is detectable mainly at short-ranges and used by natural enemies to locate aphids on a plant. It also alerts the predatory gallmidge, *A. aphidimyza*, which does not trigger the emission of (*E*)-β-farnesene and is also a prey for many aphid predators, working therefore as a synomone.
3. The emission of (*E*)-β-farnesene is regulated by aphids according to colony size and is independent of the amount of stored (*E*)-β-farnesene, aphid size and clone type. Such regulation seems to be adaptive, producing winged morphs according to colony size. However, aphids that are not attacked do not amplify their alarm signal to nearby aphids when (*E*)-β-farnesene is perceived.
4. Unlike arthropods' natural enemies that trigger the aphid alarm behaviour, entomopathogens directly affect pea aphid mothers to induce wing formation. There is no difference in the aphid wing induction between the generalist pathogen *B. bassiana* and the aphid specialist *P. neoaphidis*.

This thesis shows that (*E*)-β-farnesene emission affects and is affected by many organisms in a food web. In order to reduce ecological costs, pea aphids regulate their emission of (*E*)-β-farnesene to optimize the production of winged morphs, thus increasing the chances of successful reproduction while remaining inconspicuous to natural enemies that use (*E*)-β-farnesene as a kairomone. In addition, opportunists, such as larvae of *A. aphidimyza*, may use the ecological message associated with (*E*)-β-farnesene for their own benefit; this helps them avoid intraguild predators that also feed on aphids. The role of (*E*)-β-farnesene as a chemical signal from aphids, its emission and regulation is important to understand if we want to know how it shapes the interaction between aphids and aphids and between aphids and their natural enemies in natural ecosystems. However, even when infected by pathogens that do not trigger the alarm behaviour, pea aphids are induced to produce winged morphs independently of the pseudo crowding effect, the general mechanism triggered by arthropods' natural enemies.

This thesis provides evidence to better understand the dynamics and roles of (*E*)-β-farnesene in different interactions, but there are now new questions concerning the proximate factors and ultimate consequences of alarm pheromone emission. Future efforts should address the following questions:

- Do the amounts of (*E*)-β-farnesene emitted by pea aphids at different adult stages (pre-

reproductive, reproductive and post-reproductive) and social conditions differ?

- Although there is no chemical signal cascade in colonies to alert undisturbed aphids, is there a physical signal cascade? In other words, does the movement of alarmed aphids alert nearby undisturbed aphids that did not perceive (*E*)-β-farnesene?
- Can the regulation of (*E*)-β-farnesene emission also be affected by the predator species and their size? What are the ecological consequences of this phenomenon for predators and aphid colonies?
- Can winged offspring from an infected colony vector an entomopathogen? How is the induction of aphid wings triggered when aphids are infected? What are the benefits for entomopathogens and aphids?
- Can larvae of the predatory gallmidge, *A. aphidimyza*, also perceive direct cues of intraguild predators to avoid them?
- Can the pheromone be deactivated when exposed to the air? For how long would it still be detectable until (*E*)-β-farnesene be degraded? What environmental conditions would affect this reaction?

4. SUMMARY

For insects living in groups, chemicals are important carriers of information over different distances. Chemical communication allows insects to strategically optimize their fitness and to self-organize their activities. In the presence of a predator, insects may emit volatile organic compounds (alarm pheromones) to alert other neighbouring conspecifics of the immediate vicinity of predation. Most species of aphids make use of the sesquisterpene (*E*)-β-farnesene as the main or only component of their alarm pheromone. It is emitted with cornicle droplets that are exposed by a pair of siphunculi on the abdomen when an aphid is attacked. Besides the increase in mobility to escape an imminent risk of attack, (*E*)-β-farnesene also induces the wing formation in aphids. It increases the encounters among alarmed aphids and the tactile stimuli triggers the wing induction thus being consistent with the mechanism of wing induction when aphid populations are overcrowded. This general mechanism explains how natural enemies, even though having different strategies of attack, stimulate wing induction in the same way.

Even under natural conditions where temperature, wind and humidity may have strong influence on the response of alarm behaviour, alarmed aphids produced a higher proportion of winged offspring. However, compared with lab results, the aphid survival was much lower under field conditions, and thus there is a risk involved in responding to (*E*)-β-farnesene. The decision to search for a new plant is dependent on the environmental conditions but will also imply risks such as starvation, and fitness costs (e.g. longer developmental time and lower fecundity). In addition, (*E*)-β-farnesene also bears ecological costs such as attracting natural enemies. Aphid predators and parasitoids include a wide range of insect species that use chemical signals to locate their target. At long range, plant volatiles, especially from damaged plants, play an important role for guiding the natural enemies to patches with aphids. Once located the host plant, some natural enemies rely on chemical cues from aphids specially their alarm pheromone to find them. Therefore, (*E*)-β-farnesene is an important and high reliable cue to find host at short-range distance since it is emitted directly from aphids. It is likely that (*E*)-β-farnesene together with chemical cues from the cuticle indicates the suitability of aphids. Besides its role as pheromone for aphids and kairomone for natural enemies, (*E*)-β-farnesene also benefits the larvae of the predatory gallmidge, *Aphidoletes aphidimyza*. The larvae of this insect also are preyed by other aphid predators. When larvae perceiving (E)-β-farnesene from attacked aphids they leave the plant to avoid intraguild predation.

Since it is risky to emit (*E*)-β-farnesene (e.g. attraction of natural enemies and risk for the survival of the colony), it is likely that the emission of alarm pheromone is regulated according to the conditions aphids are in. Hence, the emission of alarm pheromone by aphids is regulated by intraspecific interactions and dependent on the colony size. If aphids are in groups, when attacked they emit less (*E*)-β-farnesene than isolated ones. However, the storage of (*E*)-β-

farnesene is not influenced by these social conditions. These emitted amounts of (*E*)-β-farnesene are sufficient to induce wing formation in colonies of respective sizes and they possibly still keep colonies undetectable to natural enemies. Perhaps for this reason aphids do not emit EBF when they perceive it.

Not all natural enemies, however, trigger the emission of alarm pheromone in aphids or depend on alarm pheromone to locate their host. Microbial pathogens, for example, are as efficient as arthropod natural enemies in killing aphids but usually forgotten to be included in the studies of aphid interactions. Two pathogens (*Pandora neoaphidis* and *Beauveria bassiana*) triggered the production of winged offspring in grouped as well as isolated aphids. There was no difference between pathogen species in their ability to induce wing formation in aphids. This indicates that the general mechanism for wing induction does not apply for entomopathogens and possibly there are other cues involved, e.g. direct disturbances in the aphid physiology or tactile stimuli from the pathogens' conidia.

5. ZUSAMMENFASSUNG

Für in Gruppen lebende Insekten sind chemische Substanzen wichtige Informationsträger über unterschiedlich weite Distanzen. Chemische Kommunikation erlaubt den Insekten, strategisch ihre Fitness zu optimieren und ihre Aktivitäten eigenständig zu organisieren. In der Gegenwart eines Feindes können Insekten flüchtige organische Substanzen (Alarmpheromone) abgeben, um andere benachbarte Artgenossen über die unmittelbare Nähe des Feindes zu informieren. Die meisten Blattlausarten nutzen das Sesquiterpen (*E*)-β-Farnesen als die Haupt- oder als die einzige Komponente ihres Alarmpheromons. Es wird in Tröpfchen, die durch ein Paar Siphone (Siphunculi) am Abdomen abgegeben werden, wenn eine Blattlaus angegriffen wird. Außer der Steigerung der Mobilität, um einem imminenten Angriffsrisiko zu entkommen, induziert (*E*)-β-Farnesen die Flügelbildung bei Blattläusen. Es erhöht das Zusammentreffen von alarmierten Blattläusen untereinander, und die taktilen Stimuli lösen die Flügelinduktion aus, ein Mechanismus, der gleichermaßen bei hohen Blattlausdichten zu beobachten ist. Dieser allgemeine Mechanismus erklärt, wie natürliche Feinde, auch wenn diese unterschiedliche Angriffsstrategien verfolgen, Flügelinduktion in der gleichen Weise stimulieren.

Alarmierte Blattläuse bringen auch unter natürlichen Bedingungen, in denen Temperatur, Wind und Feuchtigkeit die Wahrnehmbarkeit des Alarmpheromons stark herabsetzten können, verstärkt geflügelte Nachkommen zur Welt. Jedoch ist das Überleben der Blattläuse im Freiland im Vergleich zu dem im Labor viel geringer. Das Reagieren auf (*E*)-β-Farnesen im Freiland ist daher risikoreich. Die Entscheidung der Blattläuse, nach einer neuen Pflanze zu suchen, hängt von Umweltbedingungen ab, birgt jedoch Risiken wie Tod durch Verhungern oder Fitnessverlust (z.B. längere Entwicklungszeit und geringere Fertilität). Ferner ruft (*E*)-β-Farnesen ökologische Kosten, wie die Anlockung natürlicher Feinde, hervor. Blattlausprädatoren und Parasitoide sind in vielen taxonomischen Gruppen zu finden und nutzen chemische Signale, um ihre Beute oder ihren Wirt zu lokalisieren. Auf weite Entfernung spielen Pflanzenduftstoffe, vor allem von befallenen Pflanzen, eine wichtige Rolle, um die natürlichen Feinde zu den Stellen mit Blattläusen zu führen. Ist die Wirtspflanze der Blattläuse lokalisiert, können die Feinde chemischen Hinweise der Blattläuse, besonders das Alarmpheromon, nutzen, um ihre Beute zu finden. Auf kurze Entfernung ist das Alarmpheromon ist ein wichtiger und sehr verlässlicher Hinweis auf die Anwesenheit der Beute, da es direkt von Blattläusen abgegeben wird. Es ist sehr wahrscheinlich, daß (*E*)-β-Farnesen zusammen mit chemischen Hinweisen auf der Kutikula dazu dient herauszufinden, ob die Blattlaus ein passender Wirt ist. Neben der Rolle als Pheromon für Blattläuse und Kairomon für natürliche Feinde nützt (*E*)-β-Farnesen ebenso der räuberischen Gallmückenlarve, *Aphidoletes aphidimyza*. Die Larven dieser Gallmücke dienen als Beute für andere Blattlausfeinde. Wenn die Larven (*E*)-β-Farnesen der angegriffenen Blattläuse wahrnehmen, verlassen sie die Pflanze, um einer Prädation zu entgehen.

Da die Abgabe von (*E*)-β-Farnesen Risiken für die Blattläuse birgt (z.B. Anlockung natürlicher Feinde und höhere Mortalität der Kolonie), ist die Abgabe von Alarmpheromon wahrscheinlich je nach Konditionen, in denen sich Blattläuse befinden, reguliert. Die Abgabe des Alarmpheromons wird durch intraspezifische Interaktionen der Blattläuse beeinflußt und ist abhängig von der Koloniegröße. Wenn Blattläuse, die in Kolonien leben von natürlichen Feinden angegriffen werden, stoßen sie weniger (*E*)-β-Farnesen aus, als eine isolierte Blattlaus. Die von den Blattläusen gespeicherte Menge an (*E*)-β-Farnesen ist jedoch unabhängig von den sozialen Bedingungen. Die abgegebenen Mengen an (*E*)-β-Farnesen sind ausreichend, um einerseits die Flügelbildungen in Kolonien der jeweiligen Größe zu bewirken, und andererseits jedoch gering genug, um Blattlauskolonien schwer auffindbar für natürliche Feinde zu machen. Möglicherweise geben Blattläuse aus demselben Grund kein EBF ab, wenn sie es wahrnehmen.

Nicht alle natürlichen Feinde lösen jedoch die Abgabe des Alarmpheromons bei Blattläusen aus oder nutzen dieses, um ihren Wirt zu lokalisieren. Pathogene zum Beispiel verursachen eine ähnliche Mortalität bei Blattläusen wie andere natürliche Feinde aus der Gruppe der Arthropoden, werden aber oft in Studien zu Blattlaus-Interaktionen vergessen. Zwei Erreger (*Pandora neoaphidis* und *Beauveria bassiana*) rufen die Produktion von geflügelten Nachkommen sowohl in Blattlauskolonien als auch bei isolierten Blattläusen hervor. Das weist darauf hin, dass der allgemeine Mechanismus der Flügelinduktion nicht auf Entomopathogene zutrifft und wahrscheinlich andere Mechanismen, z.B. die direkte Beeinflussung der Blattlausphysiologie oder taktile Stimuli durch die Konidien der Erreger, eine Rolle spielen. Die Pathogenspezies unterscheiden sich nicht in ihrer Fähigkeit, Flügelbildung bei Blattläusen auszulösen.

6. REFERENCES

Alcock, J. (1998a). Adaptive feeding behavior. In Animal behavior: an evolutionary approach (ed. by J. Alcock), pp. 341-428. Sinauer Associates, Inc., Sunderland, Massachusetts.

Alcock, J. (1998b). The adaptive value of social living. In Animal behavior: an evolutionary approach (ed. by J. Alcock), pp. 555-602. Sinauer Associates, Inc., Sunderland, Massachusetts.

Alcock, J. (1998c). Male and female reproductive tactics. In Animal behavior: an evolutionary approach (ed. by J. Alcock), pp. 429-482. Sinauer Associates, Inc., Sunderland, Massachusetts.

Arakaki, N. (1989) Alarm pheromone eliciting attack and escape responses in the sugar-cane woolly aphid, *Ceratovacuna lanigera* (Homoptera, Pemphigidae). *Journal of Ethology*, **7**, 83-90.

Blackman, R.L. (1987). Reproduction, cytogenetics and development. In Aphids: their biology, natural enemies and control (ed. by A.K. Minks & P. Harrewijn), Vol. 2A, pp. 163-196. Elsevier, Amsterdam.

Blackman, R.L. & Eastop, V.F. (2007). Taxonomic issues. In Aphids as crop pests (ed. by H.F. van Emden & R. Harrington), pp. 1-29. CABI, Wallingford.

Blum, M.S. (1969) Alarm pheromones. *Annual Review of Entomology*, **14**, 57-80.

Bowers, W.S., Nault, L.R., Webb, R.E., & Dutky, S.R. (1972) Aphid alarm pheromone: isolation, identification, synthesis. *Science*, **177**, 1121-1122.

Braendle, C. & Weisser, W.W. (2001) Variation in escape behavior of red and green clones of the pea aphid. *Journal of Insect Behavior*, **14**, 497-509.

Brown, G.E., Godin, J.G.J., & Pedersen, J. (1999) Fin-flicking behaviour: a visual antipredator alarm signal in a characin fish, *Hemigrammus erythrozonus*. *Animal Behaviour*, **58**, 469-475.

Bruce, T.J.A., Wadhams, L.J., & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science*, **10**, 269-274.

Cardé, R.T. & Baker, T.C. (1984). Sexual communication with pheromones. In Chemical ecology of insects (ed. by W.J. Bell & R.T. Cardé), pp. 355-386. Chapman and Hall, London.

Chen, S.W. & Edwards, J.S. (1972) Observations on structure of secretory cells associated with aphid cornicles. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie*, **130**, 312-317.

de Kievit, T.R. & Iglewski, B.H. (2000) Bacterial quorum sensing in pathogenic relationships. *Infection and Immunity*, **68**, 4839-4849.

Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods: An ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1-23.

Dill, L.M., Fraser, A.H.G., & Roitberg, B.D. (1990) The economics of escape behavior in the pea aphid, *Acyrthosiphon pisum*. *Oecologia*, **83**, 473-478.

Dixon, A.F.G. (1958) The escape responses shown by certain aphids to the presence of the coccinelid *Adalia decempunctata* (L.). *Trans. R. Ent. Soc. London*, **110**, 319-334.

Dixon, A.F.G. & Agarwala, B.K. (1999) Ladybird-induced life-history changes in aphids. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266**, 1549-1553.

Edwards, J.S. (1966) Defence by smear - Supercooling in cornicle wax of aphids. *Nature*, **211**, 73-74.

Feng, M.G., Chen, C., Shang, S.W., Ying, S.H., Shen, Z.C., & Chen, X.X. (2007) Aphid dispersal flight disseminates fungal pathogens and parasitoids as natural control agents of aphids. *Ecological Entomology*, **32**, 97-104.

Fiedler, K., Holldobler, B., & Seufert, P. (1996) Butterflies and ants: The communicative domain. *Experientia*, **52**, 14-24.

Flatt, T. & Weisser, W.W. (2000) The effects of mutualistic ants on aphid life history traits. *Ecology*, **81**, 3522-3529.

Fletcher, L.E. (2007) Vibrational signals in a gregarious sawfly larva (*Perga affinis*): group coordination or competitive signaling? *Behavioral Ecology and Sociobiology*, **61**, 1809-1821.

Francis, F., Lognay, G., & Haubruge, E. (2004) Olfactory responses to aphid and host plant volatile releases: (*E*)-beta-farnesene an effective kairomone for the predator *Adalia bipunctata*. *Journal of Chemical Ecology*, **30**, 741-755.

Francis, F., Vandermoten, S., Verheggen, F., Lognay, G., & Haubruge, E. (2005) Is the (*E*)-beta-farnesene only volatile terpenoid in aphids? *Journal of Applied Entomology*, **129**, 6-11.

Frechette, B., Larouche, F., & Lucas, E. (2008) *Leucopis annulipes* larvae (Diptera : Chamaemyiidae) use a furtive predation strategy within aphid colonies. *European Journal of Entomology*, **105**, 399-403.

Greenway, A.R. & Griffith.Dc (1973) Comparison of triglycerides from aphids and their cornicle secretions. *Journal of Insect Physiology*, **19**, 1649-1655.

Greenway, A.R., Griffith.Dc, Furk, C., & Prior, R.N.B. (1974) Composition of triglycerides from aphids of 6 different families and from different seasonal forms of *Aphis evonymi*. *Journal of Insect Physiology*, **20**, 2423-2431.

Gut, J. & Vanoosten, A.M. (1985) Functional-significance of the alarm pheromone composition in various morphs of the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis Et Applicata*, **37**, 199-204.

Hales, D.F., Wilson, A.C.C., Sloane, M.A., Christophesimon, J., Legallic, J.F., & Sunnucks, P. (2002) Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids. *Genetical Research*, **79**, 203-209.

Harborne, J.B. (1993). Animal pheromones and defence substances. In Introduction to ecological biochemistry, pp. 211-242. Academic Press, London.

Harvell, C.D. (1992) Inducible defenses and allocation shifts in a marine bryozoan. *Ecology*, **73**, 1567-1576.

Hodgson, E.W., Venette, R.C., Abrahamson, M., & Ragsdale, D.W. (2005) Alate production of soybean aphid (Homoptera : Aphididae) in Minnesota. *Environmental Entomology*, **34**, 1456-1463.

Hollen, L.I. & Radford, A.N. (2009) The development of alarm call behaviour in mammals and birds. *Animal Behaviour*, **78**, 791-800.

Huang, Z.H., Feng, M.G., Chen, X.X., & Liu, S.S. (2008) Pathogenic fungi and parasitoids of aphids present in air captures of migratory alates in the low-latitude plateau of Yunnan, China. *Environmental Entomology*, **37**, 1264-1271.

Huang, Z.Y. & Robinson, G.E. (1992) Honeybee colony integration: Worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 11726-11729.

Inman, A.J. & Krebs, J. (1987) Predation and group living. *Trends in Ecology & Evolution*, **2**, 31-32.

Johnson, B. (1966) Wing polymorphism in aphids III. Influence of host plant. *Entomologia Experimentalis Et Applicata*, **9**, 213-222.

Johnson, B. & Birks, P.R. (1960) Studies on wing polymorphism in aphids, I. The developmental process involved in the production of the different forms. *Entomologia experimantalis et applicata*, **3**, 327-339.

Kats, L.B. & Dill, L.M. (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361-394.

Kelley, D.B. (2004) Vocal communication in frogs. *Current Opinion in Neurobiology*, **14**, 751-757.

Kislow, C. & Edwards, L.J. (1972) Repellent odour in aphids. *Nature*, **235**, 108-109.

Kleinjan, J.E. & Mittler, T.E. (1975) Chemical influence of ants on wing development in aphids. *Entomologia Experimentalis Et Applicata*, **18**, 384-388.

Klingauf, F.A. (1987). Feeding, adaptation and excretion. In Aphids: their biology, natural enemies and control (ed. by A.K. Minks & P. Harrewijn), Vol. 2A, pp. 225-254. Elsevier, Amsterdam.

Kunert, G., Belz, E., Simon, J.-C., Weisser, W.W., & Outreman, Y. (2010) Tell me where you live and I tell you how you behave: differences in defensive behaviour between pea aphid host races. *Ecological Entomology*, **35**, 147-154.

Kunert, G., Schmoock-Ortlepp, K., Reissmann, U., Creutzburg, S., & Weisser, W.W. (2008) The influence of natural enemies on wing induction in *Aphis fabae* and *Megoura viciae* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, **98**, 57-62.

Kunert, G. & Weisser, W.W. (2003) The interplay between density-and trait-mediated effects in predator-prey interactions: a case study in aphid wing polymorphism. *Oecologia*, **135**, 304-312.

Law, J.H. & Regnier, F.E. (1971) Pheromones. *Annual Review of Biochemistry*, **40**, 533-548.

Lees, A.D. (1966) Photoperiodic timing mechanisms in insects. *Nature*, **210**, 986-989.

Lees, A.D. (1967) Production of apterous and alate forms in aphid *Megoura viciae* Buckton with special reference to role of crowding. *Journal of Insect Physiology*, **13**, 289-318.

Lima, S.L. & Dill, L.M. (1990) Behavioral decisions made under the risk of predation - a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **68**, 619-640.

London, K.B. & Jeanne, R.L. (2003) Effects of colony size and stage of development on defense response by the swarm-founding wasp *Polybia occidentalis*. *Behavioral Ecology and Sociobiology*, **54**, 539-546.

Losey, J.E. & Denno, R.F. (1998) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. *Ecological Entomology*, **23**, 53-61.

Lucas, E. & Brodeur, J. (2001) A fox in sheep's clothing: Furtive predators benefit from the communal defense of their prey. *Ecology*, **82**, 3246-3250.

Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T., & Fuyama, Y. (2007) Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. *PLoS Biol*, **5**, 985-996.

Micha, S.G. & Wyss, U. (1996) Aphid alarm pheromone (*E*)-ß-farnesene: a host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). *Chemoecology*, **7**, 132-139.

Miyatake, T., Nakayama, S., Nishi, Y., & Nakajima, S. (2009) Tonically immobilized selfish prey can survive by sacrificing others. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 2763-2767.

Mondor, E.B., Baird, D.S., Slessor, K.N., & Roitberg, B.D. (2000) Ontogeny of alarm pheromone secretion in pea aphid, *Acyrthosiphon pisum*. *Journal of Chemical Ecology*, **26**, 2875-2882.

Mondor, E.B. & Roitberg, B.D. (2003) Age-dependent fitness costs of alarm signaling in aphids. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **81**, 757-762.

Montgomery, M.E. & Nault, L.R. (1977) Comparative response of aphids to the alarm pheromone, (*E*)-beta-farnesene. *Entomologia Experimentalis et Applicata*, **22**, 236-242.

Montgomery, M.E. & Nault, L.R. (1978) Effects of age and wing polymorphism on sensitivity of *Myzus persicae* to alarm pheromone. *Annals of the Entomological Society of America*, **71**, 788-790.

Müller, F.P. (1983) Differential alarm pheromone responses between strains of the aphid *Acyrthosiphon pisum*. *Entomologia Experimentalis Et Applicata*, **34**, 347-348.

Mustarpa, H. (1984). Olfaction. In Chemical ecology of insects (ed. by W.J. Bell & R.T. Cardé), pp. 37-72. Chapman and Hall, London.

Nault, L.R. & Bowers, W.S. (1974) Multiple alarm pheromones in aphids. *Entomologia Experimentalis Et Applicata*, **17**, 455-456.

Nault, L.R., Edwards, L.J., & Styer, W.E. (1973) Aphid alarm pheromones: secretion and reception. *Environmental Entomology*, **2**, 101-105.

Nault, L.R., Montgomery, M.E., & Bowers, W.S. (1976) Ant-aphid association - Role of aphid alarm pheromone. *Science*, **192**, 1349-1351.

Nault, L.R. & Phelan, P.L. (1984). Alarm pheromones and sociality in pre-social insects. In Chemical ecology of insects (ed. by W.J. Bell & R.T. Cardé), pp. 237-256. Chapman and Hall, London.

Nijveldt, W. (1988). Cecidomyiidae. In Aphids: their biology, natural enemies and control (ed. by A.K. Minks & P. Harrewijn), Vol. 2B, pp. 271-278. Elsevier, Amsterdam.

Nordlund, D.A. & Lewis, W.J. (1976) Termilogy of chemical releasing stimuli in intraspecific and interspecific interactions. *Journal of Chemical Ecology*, **2**, 211-220.

Oliver, T.H., Mashanova, A., Leather, S.R., Cook, J.M., & Jansen, V.A.A. (2007) Ant semiochemicals limit apterous aphid dispersal. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 3127-3131.

Pelosi, P., Zhou, J.J., Ban, L.P., & Calvello, M. (2006) Soluble proteins in insect chemical communication. *Cellular and Molecular Life Sciences*, **63**, 1658-1676.

Pickett, J.A. & Griffiths, D.C. (1980) Composition of aphid alarm pheromones. *Journal of Chemical Ecology*, **6**, 349-359.

Pike, N. & Foster, W.A. (2008). The ecology of altruism in a clonal insect. In Ecology of social evolution (ed. by J. Korb & J. Heinze), pp. 37-56. Springer, Berlin.

Polis, G.A., Myers, C.A., & Holt, R.D. (1989) The ecology and evolution of intraguild predation - Potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297-330.

Rhoden, P.K. & Foster, W.A. (2002) Soldier behaviour and division of labour in the aphid genus *Pemphigus* (Hemiptera, Aphididae). *Insectes Sociaux*, **49**, 257-263.

Robinson, G.E. (1992) Regulation of division-of-labor in insect societies. *Annual Review of Entomology*, **37**, 637-665.

Roitberg, B.D. & Myers, J.H. (1978) Adaptation of alarm pheromone responses of pea aphid *Acyrthosiphon pisum* (Harris). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **56**, 103-108.

Roy, H.E., Baverstock, J., Chamberlain, K., & Pell, J.K. (2005) Do aphids infected with entomopathogenic fungi continue to produce and respond to alarm pheromone? *Biocontrol Science and Technology*, **15**, 859-866.

Roy, H.E. & Cottrell, T.E. (2008) Forgotten natural enemies: Interactions between coccinetlids and insect-parasitic fungi. *European Journal of Entomology*, **105**, 391-398.

Roy, H.E., Pell, J.K., & Alderson, P.G. (1999) Effects of fungal infection on the alarm response of pea aphids. *Journal of Invertebrate Pathology*, **74**, 69-75.

Ruxton, G.D., Sherratt, T.N., & Speed, M.P. (2004). Signalling to predators. In Avoiding Attack - The evolutionary ecology of crypsis, warning signals, and mimicry, pp. 70-81. Oxford University Press, New York, NY.

Schneider, S.S. & McNally, L.C. (1992) Colony defense in the African honey-bee in Africa (Hymenoptera, Apidae). *Environmental Entomology*, **21**, 1362-1370.

Schwartzberg, E.G., Kunert, G., Roese, U.S.R., Gershenzon, J., & Weisser, W.W. (2008a) Alarm pheromone emission by pea aphid, *Acyrthosiphon pisum*, clones under predation by lacewing larvae. *Entomologia Experimentalis Et Applicata*, **128**, 403-409.

Schwartzberg, E.G., Kunert, G., Stephan, C., David, A., Rose, U.S.R., Gershenzon, J., Boland, W., & Weisser, W.W. (2008b) Real-time analysis of alarm pheromone emission by the pea aphid (*Acyrthosiphon pisum*) under predation. *Journal of Chemical Ecology*, **34**, 76-81.

Shah, P.A., Pickett, J.A., & Vandenberg, J.D. (1999) Responses of Russian wheat aphid (Homoptera : Aphididae) to aphid alarm pheromone. *Environmental Entomology*, **28**, 983-985.

Shaw, M.J.P. (1970a) Effects of population density on alienicolae of *Aphis fabae* Scop .1. Effect of crowding on production of alatae in laboratory. *Annals of Applied Biology*, **65**, 191-196.

Shaw, M.J.P. (1970b) Effects of population density on alienicolae of *Aphis fabae* Scop .3. Effect of isolation on development of form and behaviour of alatae in a laboratory clone. *Annals of Applied Biology*, **65**, 205-212.

Shibao, H. (1997) Social structure and the defensive role of soldiers in a eusocial bamboo aphid, *Pseudoregma bambucicola* (Homoptera : Aphididae): A test of the defence-optimization hypothesis, pp. 325-333. Springer-Verlag Tokyo, Okinawa, Japan.

Sloggett, J.J. & Weisser, W.W. (2002) Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*. *Oikos*, **98**, 323-333.

Stadler, B., Weisser, W.W., & Houston, A.I. (1994) Defense reactions in aphids - the influence of state and future reproductive success. *Journal of Animal Ecology*, **63**, 419-430.

Strong, F.E. (1967) Observations on aphid cornicle secretions. *Annals of the Entomological Society of America*, **60**, 668-673.

Su, J.W., Zhu, S.R., Zhang, Z.N., & Ge, F. (2006) Effect of synthetic aphid alarm pheromone (*E*)-beta-farnesene on development and reproduction of *Aphis gossypii* (Homoptera : Aphididae). *Journal of Economic Entomology*, **99**, 1636-1640.

Sutherland, O.R.W. (1967) Role of host plant in production of winged forms by a green strain of pea aphid *Acyrthosiphon pisum* Harris. *Nature*, **216**, 387-388.

Sutherland, O.R.W. (1969) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Physiology*, **15**, 1385-1410.

Sutherland, O.R.W. (1970) An intrinsic factor influencing alate production by two strains of pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Physiology*, **16**, 1349-1354.

Sutherland, O.R.W. & Mittler, T.E. (1971) Influence of diet composition and crowding on wing production by aphid *Myzus persicae*. *Journal of Insect Physiology*, **17**, 321-328.

Théry, M. & Heeb, P. (2008). Communication, sensory ecology, and signal evolutions. In Behavioural Ecology (ed. by É. Danchin, L.A. Giraldeau & F. Cézilly), pp. 577-612. Oxford University Press, New York, NY.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Rose, U.S.R., & Schnitzler, J.P. (2006) Practical approaches to plant volatile analysis. *Plant Journal*, **45**, 540-560.

Tollrian, R. & Harvell, C.D. (1998). The evolution of inducible defences: current ideas. In The ecology and evolution of inducible defences (ed. by R. Tollrian & C.D. Harvell), pp. 306-322. Princeton University Press, Princeton.

Tóth, E. & Duffy, J.E. (2008) Influence of sociality on allometric growth and morphological differentiation in sponge-dwelling alpheid shrimp. *Biological Journal of the Linnean Society*, **94**, 527-540.

Uematsu, K., Kutsukake, M., Fukatsu, T., Shimada, M., & Shibao, H. (2007) Altruistic defenders in a Japanese gall-forming aphid, *Quadrartus yoshinomiyai* (Homoptera : Aphididae : hormaphidinae). *Sociobiology*, **50**, 711-724.

Venzon, M., Janssen, A., Pallini, A., & Sabelis, M.W. (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. *Animal Behaviour*, **60**, 369-375.

Verheggen, F.J., Haubruge, E., De Moraes, C.M., & Mescher, M.C. (2009) Social enviroment influences aphid production of alarm pheromone. *Behavioral Ecology*, **20**, 283-288.

Visscher, P.K. (2007) Group decision making in nest-site selection among social insects. *Annual Review of Entomology*, **52**, 255-275.

Visser, J.H. & Piron, P.G.M. (1997) Odour response profiles in aphids differentiating for species, clone, form and food. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)*, **18**, 115-120.

Weisser, W.W., Braendle, C., & Minoretti, N. (1999) Predator-induced morphological shift in the pea aphid. *Proceedings of the Royal Society London Series B*, **266**, 1175-1181.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**, 249-278.

Wohlers, P. (1980) Escape responses of pea aphids, *Acyrthosiphon pisum* to alarm pheromones and additional stimuli. *Entomologia Experimentalis Et Applicata*, **27**, 156-168.

Wohlers, P. & Tjallingii, W.F. (1983) Electroantennogram responses of aphids to the alarm pheromone (*E*)-β-farnesene. *Entomologia Experimentalis Et Applicata*, **33**, 79-82.

Wright, G.A. & Schiestl, F.P. (2009) The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, **23**, 841-851.

Xiangyu, J.G., Zhang, F., Fang, Y.L., Kan, W., Zhang, G.X., & Zhang, Z.N. (2002) Behavioural response of aphids to the alarm pheromone component (*E*)-beta-farnesene in the field. *Physiological Entomology*, **27**, 307-311.

Xu, P.X., Atkinson, R., Jones, D.N.M., & Smith, D.P. (2005) *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. *Neuron*, **45**, 193-200.

Zhu, J.W., Cosse, A.A., Obrycki, J.J., Boo, K.S., & Baker, T.C. (1999) Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: Electroantennogram and behavioral responses. *Journal of Chemical Ecology*, **25**, 1163-1177.

7. ACKNOWLEDGEMENTS

I am would like to thank all the people who encouraged me during my PhD studies. I am especially thankful …

… to my doctoral adisor Wolfgang W. Weisser who gave me the opportunity to work in his group and all support to complete my experiments and this thesis.

… to Jonathan Gershenzon who supported me during my thesis and gave me permission to do the necessary research work in his group. I highly appreciate all comments and suggestion during our meetings.

…to Grit Kunert whose support, comments, guidance and discussions made this thesis possible. Thank you for making me see light when I didn't during the hard times of PhD life.

… to my colleagues and friends from the Institute of Ecology who supported me in my research work. I want to thank all for their help, support, and valuable hints. I am especially grateful to Jule, Rose, Franklin, Norma, Kerstin, Winnie, Denise, Eric, Ingrid and Sylvia for fully supporting me and for letting me try my "Deutlish".

… to Prof. Wilhelm Boland and all the members of the Department of Bioorganic Chemistry who made my time of synthesis (and the first in Jena) very productive. I would like to thank Stefan Bartram and Stefan Garms for advising during the synthesis process; Maritta Kunert and Anja David for all help with collection of volatiles; and Doreen, the Queen of Darkness, for being a true friend.

… to all members of the Department of Biochemistry for the vibrant work environment they provided. I especially would like to thank Michael Reichelt for all assistance with GC-MS. I am very grateful for Anna, Chalie, Katharina, Fred, Cristoph, John, Ina and Diana for good laughs during work.

… to my colleagues and friends in Rothamsted Research, Judy Pell, Jason Baverstock and Trish Wells. Thank you for hosting me in your lab and the great moments over tee breaks. I shall never forget the experience I had in Harpenden.

… to Conny for her great work with aphids when I did not have time. The tiger balm and cakes were essential to finish this thesis.

… to Emily Wheeler who looked closely at all my manuscripts for English style and grammar, correcting and offering suggestions for improvement.

… to all members of the weekly Boy's Night, Rohit, Martin, Maarten, Alfredo, Kusuma, Kwan, Mariana, Jan, Maripaz and Thijs. Thank you for all the pizzas, beers, bloody movies, games and funny moments. "Live long and prosper."

… to Karin Groten and Elke who gave me full support before and during my stay in Jena. There is a difference between doing a job and doing a good job. Thank you for doing an excellent job with the IMPRS.

… to my parents, brother and sister for their unconditional support to pursue what I wished.

… to my Anjo for all her love, patience and support to complete my work. Thank you for staying with me all the time.

… and finally, to God for all wonderful ecological work that he maintains.

Financial support of the International Max Planck Research School, The Max Planck Society, and the German Academic Exchange Service is gatefully acknowledged.

8. DECLARATION OF INDEPENDENT ASSIGNMENT

I declare in accordance with the conferral of the degree of doctor from the School of Biology and Pharmacy of Friedrich Schiller University Jena that the submitted thesis was written only with the assistance and literature cited in the text.

People who assisted in the experiments, data analysis and writing of the manuscripts are listed as coauthors of the respective manuscripts. I was not assisted by a consultant for doctorate theses.

The thesis has not been previously submitted whether to the Friedrich-Schiller-University, Jena or to any other university.

Jena, October 20th 2009

.....

Eduardo Hatano

9. CURRICULUM VITAE

Eduardo Hatano
Institute of Ecology, Friedrich Schiller University, Dornburger Str. 159, Jena, D-07743, Germany
Phone: (+49) 3641 949 483
Mobile: (+49) 157 7613 6046
Email: ehatano@ice.mpg.de
Webpage: imprs.ice.mpg.de/ext/homepage.html?hp=edha3500

Education and research experience

Degree	Research
Ph.D. in Ecology (candidate)	**Institute of Ecology, Friedrich Schiller University, and Max Planck Institute for Chemical Ecology, Jena, Germany** Thesis: Chemical communication in plant-aphid-predator tritrophic interactions Began April 2006 (degree expected in January 2010) Supervisor: Prof. Wolfgang W. Weisser
M.Sc. in Applied Bioscience	**Laboratory of Ecological Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo, Japan** Thesis: Eco-chemical studies on the plant-microorganism complex: Relationships between a soil-borne phytopathogen, *Aphanomyces cochlioides*, and a rhizospherous Pseudomonad *via* secondary metabolites April 2003 to March 2006 Supervisor: Prof. Emeritus Satoshi Tahara
B.Sc. in Agricultural Engineering	**Universidade Federal de Viçosa, Brazil** March 1998 to March 2003 **Laboratory of Acarology, Animal Biology Department, Universidade Federal de Viçosa, Brazil** Topic: Olfactory response of *Tetranychus evansi* (Acari: Tetranychidae) to odors from tomato plants and co-specifics August 2000 to July 2001 Supervisor: Prof. Angelo Pallini
	Laboratory of Biological Control, Biology Department, Universidade Federal de Viçosa, Brazil Topic: Biology of *Supputius cincticeps* (Heteroptera: Pentatomidae) alternating *Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Musca domestica* (Diptera: Muscidae) as food source September 1999 to July 2000 Supervisor: Prof. José Cola Zanuncio

Presentations and posters

- **Presentations**
  - **Hatano E.**, Kunert G., Weisser W.W. (2009) The role of the alarm pheromone for pea aphid wing induction under field conditions. *German Entomological Society Meeting*, Göttingen, Germany, March.
  - **Hatano E.**, Deora A., Hashidoko Y., Fukushi Y., Satoshi T. (2006) Isolation of anti-Peronosporomycete γ-lactone derivatives from *Pseudomonas* sp. EC-S101. *Japan Society for Bioscience, Biotechnology, and Agrochemistry*, Kyoto, Japan, April.
  - **Hatano, E.** Sarmento, R.A., Oliveira, E.E. and Pallini, A. (2002) Infochemicals from the complex tomato plant-herbivore attract the predator *Cycloneda sanguinea* (Coleoptera: Coccinelidae)?. *III Brazilian Ecological Chemistry Meeting*, Campinas, Brazil, December.

- **Hatano E.**, Costa L.R.T., Oliveira C.L., Oliveira E.E., Sarmento R.A., Pallini A. and Lima, E.R. (2002) Can *Cycloneda sanguinea* (Coleoptera: Coccinelidae) behavior be changed by the presence of *Eriophis connexa* (Coleoptera: Coccinelidae) on injured tomato plants?. *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
- **Hatano E.** and Pallini A. (2001) Olfactive response of *Tetranychus evansi* (Acari: Tetranychidae) to tomato plants and co-specifics odors. *Scientific Initiation Symposium*, Viçosa, Brazil, February.
- **Hatano E.**, Santos G.A. dos and Zanuncio J.C. (2000) Biology of *Supputius cincticeps* (Heteroptera: Pentatomidae) in alternated feeding with *Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Musca domestica* (Diptera: Muscidae). *X Scientific Initiation Symposium*, Viçosa, Brazil, December.

- **Posters**

  - **Hatano E.**, Kunert G., Kunert M., David A., Gershenzon J. and Weisser W.W. (2009) Don't talk loudly: The emission of alarm pheromone from pea aphids regulated by group-living conditions. *25th Annual Meeting of the International Society of Chemical Ecology*, Neuchâtel, Switzerland, August.
  - **Hatano E.**, Kunert G., Bartram S., Boland W., Gershenzon J. and Weisser W.W. (2007) Chemical response of aphids to alarm pheromone: dynamics of (*E*)-β-farnesene emission from aphid colonies. *Ecology of Aphidophaga 10*, Athens, Greece, September.
  - Deora A., **Hatano E.**, Hashidoko Y., Fukushi Y., Tahara S. (2006) Antifungal and hyphal branch-inducing properties and principles of *Pseudomonas jessenii* strain EC-S101 against *Aphanomyces cochlioides* AC-5. *11th IUPAC International Congress of Pesticide Chemistry*, Port Island, Kobe, Japan, August.
  - Oliveira C.L., Oliveira E.E., **Hatano E.**, Costa L.R.T., Sarmento R.A., Euzebio D.E. and Pallini A. (2002) Changes on feeding of the predator *Cycloneda sanguinea* (Coleoptera: Coccinellidae) can affect its offspring?. *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - Euzebio D.E., Oliveira C.L., Oliveira E.E., **Hatano E.**, Costa L.R.T., Sarmento R.A. and Pallini A. (2002) Biological aspects of *Cycloneda sanguinea* (Coleoptera: Coccinelidae) fed with *Myzus persicae* (Homoptera: Aphididae). *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - Costa L.R.T., **Hatano E.,** Oliveira E.E., Oliveira C.L., Tuelher E.S., Sarmento R.A. and Pallini A. (2002) Chemical communication between the predator *Cycloneda sanguinea* (Coleoptera: Coccinellidae) and plants infested with spider mites. *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - Oliveira E.E., **Hatano E.**, Oliveira C.L., Costa L.R.T., Chaves F.F., Sarmento R.A., Pallini A. and Lima E.R. (2002) Odours from tomato plants damaged by the red spider mite *Tetranychus evansi* (Acari: Tetranychidae) mediating competition between two generalist predators. *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - **Hatano E.**, Costa L.R.T., Oliveira C.L., Oliveira E.E., Sarmento R.A. and Pallini A. (2002) Can undamaged plants near damaged ones become more resistant to the herbivore *Tetranychus evansi* (Acari: Tetranychidae). *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - Tuelher E.S., Chaves F.F., Ferreira J.A.M., **Hatano E.**, Oliveira E.E. and Pallini A. (2002) Response of coffee plants induced by *Oligonychus ilicis*. *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.
  - Costa L.R.T., **Hatano E.** Oliveira E.E., Oliveira C.L., Sarmento R.A. and Pallini A. (2002) Olfactive response of the predator *Cycloneda sanguinea* (Coleoptera: Coccinellidae) to tomato plants infested with *Myzus persicae* (Homoptera: Aphididae). *XII Scientific Initiation Symposium*, Viçosa, Brazil, November.

Personal information

- **Nationality:** Brazilian
- **Date of birth:** July 2, 1980
- **Place of birth:** São Bernardo do Campo, São Paulo

10. LIST OF PUBLICATIONS

- **Journal Manuscripts**
  - Hosseini M., **Hatano E.**, Ashouri A., Weisser W.W. (2009) Aphid alarm pheromone mediates avoidance of habitats by predatory gallmidge *Aphidoletes aphidimyza.* Ecological Entomology. Submitted.
  - **Hatano E.**, Kunert G., Weisser W.W. (2009) Aphid wing induction and ecological costs of alarm pheromone emission under field conditions. PLoS ONE. Accepted.
  - Deora A., **Hatano E.**, Satoshi T., Yasuyuki H. (2009) Inhibitory effects of furanone metabolites of a rhizobacterium, *Pseudomonas jessenii*, on phytopathogenic *Aphanomyces cochlioides* and *Pythium aphanidermatum*. Plant Pathology. *In Press*.
  - **Hatano E.**, Kunert G., Michaud J.P. and Weisser W.W. (2008) Chemical cues mediating aphid location by natural enemies. *Eur. J. Entomol.* 105, 797–806.
  - **Hatano E.**, Kunert G., Bartram S., Boland W., Gershenzon J. and Weisser W.W. (2008) Do aphid colonies amplify their alarm pheromone emission? *J. Chem. Ecol.*, 34, 1149-1152.
  - **Hatano E.**, Hashidoko Y., Deora A., Fukushi Y., Tahara S. (2007) Isolation and structure elucidation of Peronosporomycetes hyphal branching-inducing factors Produced by *Pseudomonas jessenii* EC-S101. *Biosci. Biotech. Biochem*., 71, 1601-1605.
  - de Oliveira C.R.F., Matos C.H.C., **Hatano E.** (2007) Occurrence of *Pyemotes* sp. on *Tuta absoluta* (Meyrick). *Braz. Arch. Biol. Technol.*, 50, 929-932.
  - Moreira L.R., Oliveira E.E., **Hatano E.**, Pallini A., Vilela E.F., Lima F.N.B. (2004) Do infochemicals from tomato plants influence herbivores behaviour?. *Revista Ceres*, 51, 397-404.

- **Book chapters**
  - Pallini A., Matos C.H.C., **Hatano E.** and Venzom M. (2002) Integrated pest management of mites in tropical and subtropical fruit plants. In: *Integrated management: tropical fruit plants – diseases and pests.* Ed. Viçosa: Imprensa universitaria, v. 4, 579-614.

- **Conference Proceedings**
  - **Hatano E.**, Deora A., Tahara S., and Hashidoko Y. (2007) γ-Lactone derivatives from a beneficial rhizobacterium, *Pseudomonas jessenii* EC-S101, regulate rhizo-biocomplex. In: *Sustainable Agriculture for Food, Bio-energy and Livelihood Security*. D.P. Singh, V.S. Tomar, R.K. Behl, S.D. Upadhyaya, M.S. Bhale, D. Khare (eds.), pp 488-498, Agrobios (International), Jodhpur, India.
  - **Hatano E.** Oliveira E.E., Pallini A. and Silva D.J.H. da (2002) Olfactive response of the red spider mite to tomato plant odours. In: *42º Brazilian Olericulture Conference and 11º Latin-American Horticulture Conference*, Uberlandia, August, p.384.

- **Newspaper Article**
  - Santos F.A.A. and **Hatano E.** (1999) Biological control, Tribuna de Alagoas, 843, June 5, 1999.